

# EXHIBIT 21

**United States Patent** [19][11] **4,032,637****Spiegel et al.**[45] **June 28, 1977**[54] **METHOD OF PROMOTING SLEEP**

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[22] **Filed:** Oct. 31, 1974

[21] **Appl. No.:** 519,570

**Related U.S. Application Data**

[63] Continuation of Ser. No. 399,076, Sept. 20, 1973,  
 abandoned.

[30] **Foreign Application Priority Data**

Sept. 26, 1972 Switzerland ..... 14031/72

[52] **U.S. Cl.** ..... 424/247

[51] **Int. Cl.<sup>2</sup>** ..... A61K 31/54

[58] **Field of Search** ..... 424/247

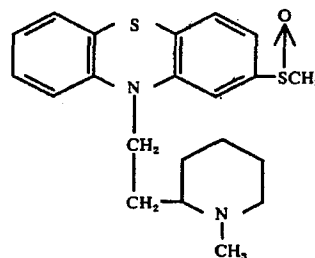
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[57] **ABSTRACT**

The present invention concerns a novel therapeutic use  
 as a sleep-promoting agent for the known pharmaceuti-  
 cal compound 10-[2-(1-methyl-2-piperidyl)ethyl]-2-  
 methylsulphonylphenothiazine of the formula:



**5 Claims, No Drawings**

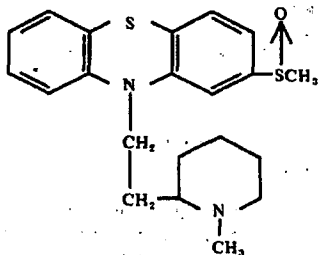
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**METHOD OF PROMOTING SLEEP**

This is a continuation of application Ser. No. 399,076 filed Sept. 20, 1973 now abandoned.

The present invention relates to a novel therapeutic use for the known pharmaceutical compound, 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonyl-phenothiazine of formula I,



Production of the compound of formula I is disclosed, e.g. in U.S. Pat. No. 3,084,161. The compound is also known under the name Mesoridazine, and is at present indicated for use in psychiatry for the treatment of chronic schizophrenia, acute schizophrenia, alcoholism, behaviour disorders, psychically retarded patients with psychotic behaviour disorders, various psychoses, e.g. infantile and juvenile psychoses, and organic psychosyndromes with circulatory disorders.

It has now been found that the compound is useful as a sleep-promoting agent, e.g. for the treatment of severe or moderate sleep disorders, as indicated by the following tests:

In double blind clinical tests with patients between 21 and 68 years old, the compound was administered p.o. at a daily dose of 10 mg and 20 mg.

Sleep-promoting activity similar to that of known sleep-promoting agents was determined subjectively on statistical evaluation of the results of a questionnaire, concerning the time taken to fall asleep, quality of sleep, duration of sleep, frequency of waking up from sleep and condition in the morning. It was found that the sleep-promoting activity at doses of 10 mg was similar to the sleep-promoting activity at doses of 20 mg. However, incidence of hangover on the morning after, generally experienced after administration of a sleep-promoting agent, was observed only at higher doses and was in general weak and insignificant. Therefore the compound is indicated especially for use in nongeriatric patients who lead active lives and are particularly susceptible to the effects of such hangovers.

In electrophysiological tests with female patients aged between 50 and 60 years old, on p.o. administration of 10 mg of the compound daily, two sleep phases were detected:

the NREM (orthodox or slow wave) sleep, and the REM (paradoxical) sleep. The deepness of sleep increases in the NREM phase; a differentiation is made between stages 1, 2, 3 and 4.

Although the function of sleep has not been elucidated in detail, there is good evidence for the assumption that the REM sleep and the NREM sleep fulfil different functions: in stages 3 and 4 of the NREM sleep, a substantial portion of growth hormone is liberated, whereas during the course of the REM sleep phase, synthetic processes, especially in the brain, take place.

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Generally on administration of known sleep-promoting agents, the REM sleep phase is proportionally reduced. When use of the sleep-promoting agent is stopped, a rebound reaction occurs which is associated with vivid dreams and nightmares.

Many sleep-promoting agents, e.g. benzodiazepines, decrease substantially stages 3 and 4 of the NREM phase after chronic intake.

However, on administration of the present compound of formula I only an insignificant distortion of the normal electrophysiological sleep pattern was determined with no or little suppression of REM and stages 3 and 4 sleep.

The above clinical tests indicate that the present compound of formula I is well tolerated as a sleep-promoting agent by patients.

It will be appreciated that sleep-promoting activity in a phenothiazine derivative is especially interesting as such derivatives are not known to be drugs of dependence or abuse.

For the sleep-promoting use, the dosage will, of course, vary depending on the mode of administration and condition to be treated. However, in general satisfactory results are obtained when administered at a daily dosage of from about 0.05 to about 0.5 mg per kg. animal body weight, conveniently administered shortly before the normal time of sleep. For the larger mammals, the total daily dosage is in the range of from about 5 to about 25 mg, preferably from about 10 to about 20, especially from about 10 to about 15 mg, preferably administered perorally.

The compound of formula I may be administered in pharmaceutically acceptable acid addition salt form. Such acid addition salt forms exhibit the same order of activity as the free base form. Suitable acids for salt formation include hydrochloric, hydrobromic and sulphuric acids and maleic, acetic and methane sulphonic acids. The preferred pharmaceutically acceptable acid addition salt form is however the besylate salt form. Pharmaceutical compositions containing the compound of formula I in free base form or in pharmaceutically acceptable acid addition salt form, in association with a pharmaceutical carrier or diluent are known and may be prepared in conventional manner. For example, the compound may be worked up in known manner together with the usual suitable pharmaceutical adjuvants, to produce the conventional solid or liquid galenical preparations for oral, rectal or parenteral administration, e.g. tablets, capsules, dragées, drop solutions, syrups, suppositories or sterile solutions. The compound may be mixed with the adjuvants in known manner and worked up into a dose form. A preferred dose form is a solid preparation suitable for oral administration, e.g. tablets, capsules or dragées. Examples of suitable adjuvants and carrier materials for solid forms are: talc, lactose, sucrose, maize starch, polyvinyl pyrrolidone, magnesium stearate, dimethyl silicone oil, polyethylene glycol, silicic acid, stearic acid, microcrystalline cellulose and gelatine. The solid preparations may contain between 5 and 25 mg per unit dose. Suitable dose forms contain, e.g., 5, 10 or 15 mg of active compound. The pharmaceutical compositions may also contain suitable preserving, stabilizing or wetting agents, solubilizers, sweetening or colouring agents and flavourings.

The following Examples set out details of pharmaceutical compositions suitable for use in the method of the invention, it being understood that further prepara-

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tions, such as those known in the literature, suitable for use in the method of the invention may be prepared by conventional techniques.

**EXAMPLE 1: Tablets containing 5 mg of active compound If**

EXAMPLE 1: Tablets containing 5 mg of active compound	
Mesoridazine besylate*	7.05 mg
dimethyl silicone oil	0.50 mg
polyethylene glycol	0.50 mg
polyvinyl pyrrolidone	3.00 mg
sucrose powder	3.00 mg
talc	3.00 mg
maize starch	6.00 mg
lactose	26.95 mg
for a tablet of	50.00 mg

The active compound is mixed in the usual manner with the above adjuvants and carrier materials, and the mixture is granulated and pressed into tablets in known manner.

**EXAMPLE 2: Tablets containing 10 mg of active compound**

Example 2: Tablets containing 10 mg of active compound	
Mesoridazine besylate*	14.10 mg
silicic acid	0.10 mg
stearic acid	0.70 mg
microcrystalline cellulose	5.00 mg
talc	1.50 mg
maize starch	2.60 mg
lactose	30.50 mg
gelatine	0.50 mg
for a tablet of	55.00 mg

\*corresponding to 10 mg of Mesoridazine base

Admixture of the single components, granulation and tabletting may be effected in known manner.

**EXAMPLE 3: Dragées containing 5 mg of active compound**

Dragées may be produced in known manner from the tablets described in Example 1, using a suitable dragée coating.

**EXAMPLE 4: Dragées containing 10 mg of active compound**

Dragées may be produced in known manner from the tablets described in Example 2, using a suitable dragée coating.

**EXAMPLE 5: Capsules containing 5 mg of active compound**

EXAMPLE 5: Capsules containing 5 mg of active compound	
Mesoridazine besylate	7.05 mg
lactose pulverized	30.00 mg
lactose crystalline	116.00 mg
talc siliconized	6.95 mg
for a capsule content of	160.00 mg

The components are mixed together, the mixture is sieved and filled into capsules.

**EXAMPLE 6: Capsules containing 10 mg of active compound**

EXAMPLE 6: Capsules containing 10 mg of active compound	
Mesoridazine besylate	14.10 mg
lactose	302.90 mg
talc	13.00 mg
for a capsule content of	330.00 mg

The components are mixed together, the mixture is sieved and filled into capsules.

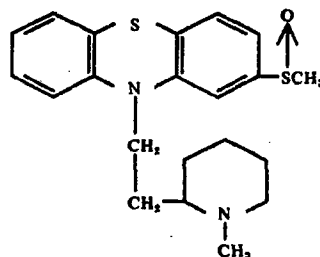
**EXAMPLE 7: Capsules containing 15 mg of active compound**

EXAMPLE 7: Capsules containing 15 mg of active compound	
Mesoridazine besylate	21.15 mg
lactose pulverized	75.00 mg
lactose crystalline	285.00 mg
talc siliconized	18.85 mg
for a capsule content of	400.00 mg

The components are mixed together, the mixture is sieved and filled into capsules.

We claim:

1. A method of promoting sleep in animals, which comprises administering to an animal suffering from insomnia a sleep promoting effective dose of 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonylphenothiazine of formula:

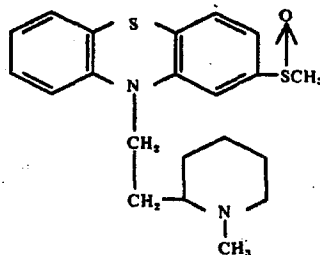


in free base form or in pharmaceutically acceptable acid addition salt form thereof.

2. A method according to claim 1, which comprises administering 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonylphenothiazine at a daily dose of from about 0.05 to about 0.5 mg/kg animal body weight.

3. A method according to claim 2, which comprises administering 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonylphenothiazine at a daily dose of from about 5 to about 25 mg.

4. A method of promoting sleep in mammals, which comprises administering to a mammal suffering from insomnia a daily dose of from about 10 to 20 mg. of 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonylphenothiazine of the formula:



in free base form or in pharmaceutically acceptable acid addition salt form thereof.

5. A method according to claim 4, which comprises administering 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylsulphonylphenothiazine at a daily dose of from about 10 to 15 mg.

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# EXHIBIT 22

**United States Patent** [19]

Treiber et al.

[11] **4,336,263**[45] **Jun. 22, 1982**

[54] **1,4-CYCLOALKANO-OXAZEPINES, SALTS THEREOF AND ANALGESIC USES THEREOF**

[75] Inventors: **Hans J. Treiber, Bruehl; Dieter Lenke, Ludwigshafen; Wolfgang Worstmann, Gruenstadt, all of Fed. Rep. of Germany**

[73] Assignee: **BASF Aktiengesellschaft, Fed. Rep. of Germany**

[21] Appl. No.: **193,947**

[22] PCT Filed: **Oct. 22, 1979**

[86] PCT No.: **PCT/EP79/00080**

§ 371 Date: **Jun. 26, 1980**

§ 102(e) Date: **Jun. 16, 1980**

[87] PCT Pub. No.: **WO80/00838**

PCT Pub. Date: **May 1, 1980**

[30] **Foreign Application Priority Data**

Oct. 26, 1978 [DE] Fed. Rep. of Germany ..... 2846567

[51] Int. Cl.<sup>3</sup> ..... **C07D 267/14; C07D 267/12; A61K 31/395**

[52] U.S. Cl. .... **424/244; 260/330.8**

[58] Field of Search ..... **260/333**

[56] **References Cited**

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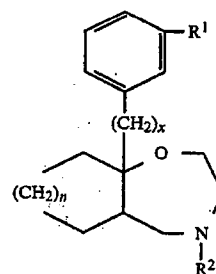
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*Primary Examiner*—Norma S. Milestone  
*Attorney, Agent, or Firm*—Keil & Witherspoon

[57]

**ABSTRACT**

The invention relates to novel 1,4-cycloalkano-oxazepines of the general formula



where R<sup>1</sup> is hydrogen, hydroxyl or alkoxy or acyloxy of 1 to 4 carbon atoms, R<sup>2</sup> is a hydrocarbon radical of 1 to 3 carbon atoms, n is 1, 2 or 3 and x is 0 or 1, and of its salts with physiologically acceptable acids; processes for their preparation, and their use in therapy.

The novel substances are suitable for the pharmacotherapy of pains of various origins.

**11 Claims, No Drawings**

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# 1,4-CYCLOALKANO-OXAZEPINES, SALTS THEREOF AND ANALGESIC USES THEREOF

## TECHNICAL FIELD

The invention relates to novel 1,4-cycloalkano-oxazepines, processes for their preparation and their use in combating pain (algias).

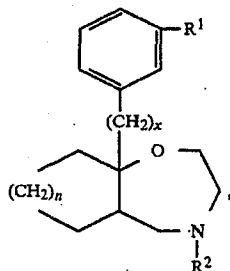
## PRIOR ART

German Pat. No. 1,620,198 discloses that certain benzoxazocines have an analgesic action. The best-known of these compounds is Nefopam (5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-benz[f]-2,5-oxazocine).

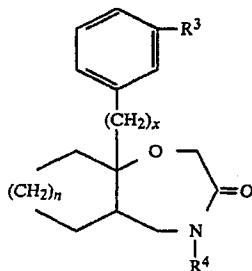
## DESCRIPTION OF INVENTION

We have found compounds which are substantially more active than Nefopam.

The present invention relates to 1,4-cycloalkano-oxazepines of the general formula I



where R<sup>1</sup> is hydrogen, hydroxyl or alkoxy or acyloxy of 1 to 4 carbon atoms, R<sup>2</sup> is a hydrocarbon radical of 1 to 3 carbon atoms, n is 1, 2 or 3 and x is 0 or 1, and of their salts with physiologically acceptable acids. The invention also relates to a process for the preparation of the 1,4-cycloalkano-oxazepines of the general formula I, wherein a compound of the general formula II



where R<sup>3</sup> is hydrogen or alkoxy of 1 to 4 carbon atoms and R<sup>4</sup> is a hydrocarbon radical of 1 to 3 carbon atoms or is benzyl, is reduced, after which, where appropriate, alkoxy is replaced by hydroxyl or acyloxy and/or benzyl is replaced by a hydrocarbon radical and, if desired, the resulting compound is converted to a salt with a physiologically acceptable acid.

Finally, the invention also relates to pharmaceuticals containing 1,4-cycloalkano-oxazepines of the general formula I and to the use of the novel substances in combating pain (algias).

The compounds I contain two asymmetric carbon atoms, so that they can in principle form two diastereomeric series. The present invention only relates to

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those compounds in which the two rings are cis-linked to one another. The novel compounds can be prepared in the form of their racemates and in the form of their antipodes.

A strong reducing agent, eg. diborane or, preferably, lithium aluminum hydride, is required for reducing a compound II. Particularly suitable solvents are tetrahydrofuran, dimethoxyethane, dioxane and ether.

The reduction takes place at an elevated temperature, preferably at the boiling point of the solvent.

A benzyl radical in the 4-position can easily be removed by catalytic hydrogenation. The hydrogenation may be carried out in an alcohol, eg. methanol or ethanol, or in acetic acid as the solvent, preferably at room temperature, and using a noble metal of main group 8 of the periodic table, preferably Pd/charcoal, as the catalyst.

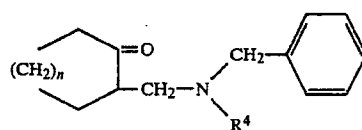
The resulting secondary amine can be alkylated by means of an alkyl halide in the presence of an acid acceptor, such as an alkali metal carbonate, alkali metal hydroxide or tertiary amine, in a solvent such as methyl isobutyl ketone, at about 0°-150° C., preferably at about 80° C. The alkylation can also be effected by reaction with a carboxylic acid followed by reduction of the C=O group with lithium aluminum hydride.

The replacement of methoxy by hydroxyl may be carried out with, for example, sodium methylmercaptide, in a dipolar aprotic solvent, eg. hexamethylphosphorotriamide, dimethylsulfoxide or dimethylformamide, at 50°-200° C., preferably 80°-150° C.

Virtually all conventional methods may be employed for acylating the free hydroxyl groups. The simplest method is to react such groups with an acid anhydride or acid halide at an elevated temperature.

The starting materials of the general formula II, required for the preparation of the novel compounds, have not previously been described. They may be prepared as follows:

A Mannich compound of the formula III is prepared from a cycloalkanone, formaldehyde and a secondary benzylamine derivative (cf. Organic Reactions I (1942), British Pat. No. 615,136, J. Chem. Soc. 1950, 1512 and J. Org. Chem. 24, (1959), 1069):

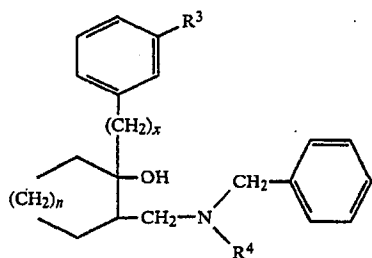


Reaction of III with an appropriate Grignard compound gives the compound IV (cf. J. Am. Chem. Soc. 71 (1949), 2050, British Pat. No. 997,399, German Pat. No. 1,199,764 and Arzneim. Forsch. 28 (1978), 107):

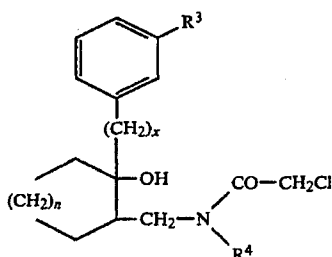
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from which the benzyl radical is removed by hydrogenation. Reaction of the resulting compound with chloroacetyl chloride in the presence of dilute sodium hydroxide solution or triethylamine gives the compound V



The compound II may then be prepared from V by heating with a base, eg. potassium tert.-butanolate, in dimethylsulfoxide.

In compound V, the free OH group and the  $\text{CH}_2\text{—NR}^4\text{—CO—CH}_2\text{Cl}$  group are in the cis-position relative to one another, if the compound is prepared by the above method.

If it is desired to prepare compound I in the form of its optical antipodes, it is advantageous to separate the racemates at the stage of compound IV.

The compounds according to the invention exhibit a pronounced analgesic action.

The model used for testing the analgesic action was the tail flick test of D'AMOUR and SMITH (J. Pharmacol. 72 (1941), 74–79). In this experiment, the compounds to be tested were administered intraperitoneally or orally, as aqueous solutions (injection volume 10 ml/kg) to groups of 10 female mice (NMRI strain) weighing 20–22 g each.

Pain reactions are caused before, and 30 minutes after, the administration of the compound, by means of

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IV

thermal irritation (the tail being exposed to focused heat radiation from a halogen lamp for at most 30 seconds).

The time until the tail is drawn out of the radiation zone by reflex action is measured, and taken as the reaction time. Its average value, for 670 untreated animals, is  $6.5 \pm 0.29$  sec.

Analgesic substances increase the reaction time, to an extent depending on the dose. There is a linear relationship between the logarithms of the doses (mg/kg) and of the relative increase in reaction time ( $\Delta\%$ ), from which the dose, defined as the ED 100%, which doubles the reaction time can be calculated by means of regression analysis. For a radiation exposure period of at most 30 seconds, the maximum possible increase in the reaction time is about 360%.

A dose-dependent analgesic action is demonstrable both after intraperitoneal and after oral administration (Table 1). The novel substances are, in this test, substantially superior to the known analgesic Nefopam (5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine), particularly using oral administration, namely the pharmacotherapeutically important route of administration. The activity of the compounds according to the invention is from about 2 to 5 times greater. The greater oral activity is accompanied by an increase in the relative enteral activity, this being a parameter of exceptional importance with regard to safety of use. The enteral activity is expressed as the quotient of the effective doses (ED 100%) for intraperitoneal and oral administration. It is from 0.35 to 0.96 for the novel compounds and is thus from 2.1 to 5.6 times greater than the enteral activity of Nefopam.

Under the conditions of the tail flick test, the reaction time can be increased by the compounds according to the invention, using either route of administration, the maximum increase without causing fatalities due to toxic effects of the compounds being 275–416% for intraperitoneal administration and 280–297% for oral administration. In contrast, with Nefopam the maximum possible increases are only 192% (21.5 mg/kg administered intraperitoneally) and 93% (46.4 mg/kg administered orally). Higher doses (46.4 mg/kg administered intraperitoneally or 100 mg/kg administered orally) already prove lethal and kill 60% of the treated animals (Table 1).

The comparatively low toxicity of the novel compounds can also be deduced from the acute lethal dose (LD 50) for intraperitoneal administration (Table 2). The LD 50 values are from about 90% to 240% higher than those of Nefopam.

TABLE 1

Compound from Example No.	Intraperitoneal administration				Oral administration				
	ED 100% <sup>(1)</sup>	relative activity	Maximum action <sup>(2)</sup>		ED 100%	relative activity	Maximum action <sup>(2)</sup>		Q <sup>(3)</sup>
			mg/kg	$\Delta\%$			mg/kg	$\Delta\%$	
3	11.4	0.71	46.4	416	17.0	2.73	100	397	0.67
6	4.66	1.73	46.4	380	9.49	4.89	46.4	364	0.49
7	6.55	1.23	46.4	364	18.6	2.50	46.4	332	0.35
9 (–)	11.0	0.74	46.4	348	17.6	2.64	100	397	0.63
9 (+)	16.7	0.48	46.4	249	23.5	1.97	100	370	0.71
7 $\beta$	12.6	0.64	46.4	275	13.1	3.54	100	280	0.96

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TABLE 1-continued

Compound from Example No.	Intraperitoneal administration				Oral administration				Q <sup>(3)</sup>
	ED 100% <sup>(1)</sup>	relative activity	Maximum action <sup>(2)</sup> mg/kg	$\Delta\%$	ED 100%	relative activity	Maximum action <sup>(2)</sup> mg/kg	$\Delta\%$	

<sup>(1)</sup>Dose in mg/kg which increases the reaction time by 100%<sup>(2)</sup>Maximum increase in reaction time for a dosage step of  $\sqrt[3]{10}$ <sup>(3)</sup>Q = enteral activity =  $\frac{\text{ED 100\% for intraperitoneal administration}}{\text{ED 100\% for oral administration}}$ <sup>(4)</sup>6 out of 10 animals died at 46.4 mg/kg<sup>(5)</sup>6 out of 10 animals died at 100 mg/kg

Nefopam	8.08	$\approx 1.00$	21.5 <sup>(4)</sup>	192	46.4	1.00	46.4 <sup>(5)</sup>	93	0.17
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TABLE 2

Compound of Example No.	LD 50 mg/kg	Relative toxicity
3	110	0.52
6	127	0.45
7	165	0.35
9 (-)	145	0.40
9 (+)	110	0.52
7 $\beta$	194	0.30
Nefopam	57.3	$\approx 1.00$

## COMMERCIAL UTILITY

The novel compounds are suitable for the pharmacotherapy of pains of various origins. They may be administered orally or parenterally (intravenously or intramuscularly) in the conventional manner.

The dosage depends on the age, condition and weight of the patient and on the route of administration. As a rule, the daily dose of active compound is from about 0.1 to 2.0 mg/kg of body weight for oral administration and from about 0.05 to 1.0 mg/kg of body weight for parenteral administration. In a normal case, satisfactory results are achieved with a daily dose of from 0.3 to 1.5 mg/kg administered orally or from 0.1 to 0.5 mg/kg administered parenterally.

The novel compounds may be used in one of the solid or liquid conventional galenical forms for administration, for example as tablets, capsules, powders, granules, dragees, solutions or suppositories. These are prepared in the conventional manner by formulating the active compound together with the conventional galenical auxiliaries, such as tablet binders, fillers, preservatives, tablet-disintegrating agents, flow regulators, plasticizers, wetting agents, dispersants, emulsifiers, solvents, retarders and/or antioxidants (cf. L. G. Goodman and A. Gilman: The Pharmacological Basis of Therapeutics).

The novel compounds can also be administered in the form of their salts with physiologically acceptable acids. Examples of such acids are hydrochloric acid, sulfuric acid, phosphoric acid, tartaric acid, citric acid, fumaric acid, acetic acid, formic acid, succinic acid, maleic acid, lactic acid and amidosulfonic acid.


## PREPARATION OF THE STARTING MATERIALS

## (a) 2-(N-Benzylmethylamino)-methyl-cyclopentanone hydrochloride

87.5 g (0.55 mole) of N-benzylmethylamine hydrochloride, 84 g (1.0 mole) of cyclopentanone, 45 g of paraformaldehyde ( $\Delta 1.5$  moles of formaldehyde) and 500 ml of ethanol are refluxed for one hour whilst stirring, during which solution occurs. After the solution has cooled, the solvent is distilled off under reduced

pressure, the oily residue is dissolved in 100 ml of isopropanol and 500 ml of ethyl acetate are then added, whereupon the product crystallizes out after some time. Yield 75 g (54% of theory). Melting point 129°-130° C.

The following compounds were obtained in a similar manner:

n	R	Melting point of the hydrochloride (°C.)	Yield
2	CH <sub>3</sub>	140	83%
2		250-251	48%
3	CH <sub>3</sub>	120-123	43%

(b)

## 2-(N-Benzylmethylamino)-methyl-1-(3-methoxybenzyl)cyclohexanol

67 g (0.25 mole) of 2-(N-benzylmethylamino)methylcyclohexanone hydrochloride obtained as described in (a) are introduced in the course of 30 minutes, whilst stirring and cooling, into a Grignard solution which has been prepared from 110 g (0.7 mole) of 3-methoxybenzyl chloride, 16.8 g of magnesium and 700 ml of dry ether; stirring is continued for 16 hours at room temperature, the reaction mixture is decomposed with an excess of concentrated ammonium chloride solution, the ether layer is separated off and dried with sodium sulfate, the ether is driven off and the residue is distilled under reduced pressure.

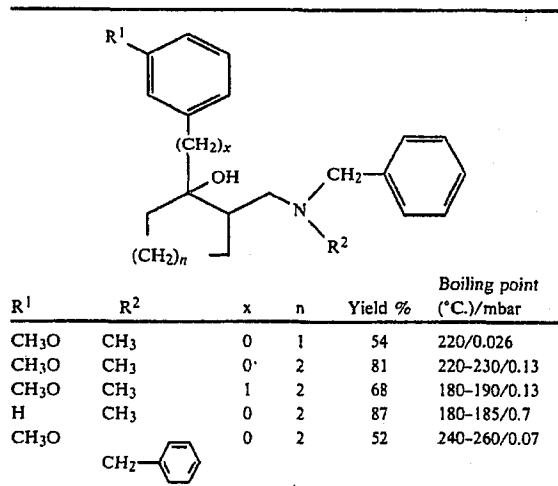
Yield: 60 g (68% of theory). Melting point 200°-205° C./0.007 mbar.

The following compounds were prepared in a similar manner:

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Resolution of the racemate of  
2-(N-benzylmethylamino)methyl-1-(3-methoxy-phenyl)-cyclohexanol

A solution of 150 g (0.44 mole) of 2-(N-benzylmethylamino)-methyl-1-(3-methoxy-phenyl)-cyclohexanol and 168 g (0.44 mole) of L(-)-O,O-dibenzoyltartaric acid monohydrate in 1,000 ml of isopropanol is prepared; from this, the dibenzoyltartrate of the levo-rotatory base crystallizes out. After recrystallizing this material twice from a 3-fold amount of isopropanol, 130 g (86% of theory) of a product is obtained which shows no change in optical rotation after further recrystallizations.

Specific optical rotation:  $[\alpha]_D^{20} = -77^\circ$  (methanol, C=27 mg/ml).

The salt is converted in the conventional manner to the base, having the following specific optical rotation:  $[\alpha]_D^{20} = -89^\circ$  (methanol, C=22 mg/ml).

If D(+)-O,O-dibenzoyltartaric acid is used for the resolution, the dibenzoyltartrate of the dextrorotatory base, having a specific optical rotation  $[\alpha]_D^{20} = +77^\circ$ , and the base, having a specific optical rotation  $[\alpha]_D^{20} = +89^\circ$ , are obtained similarly.

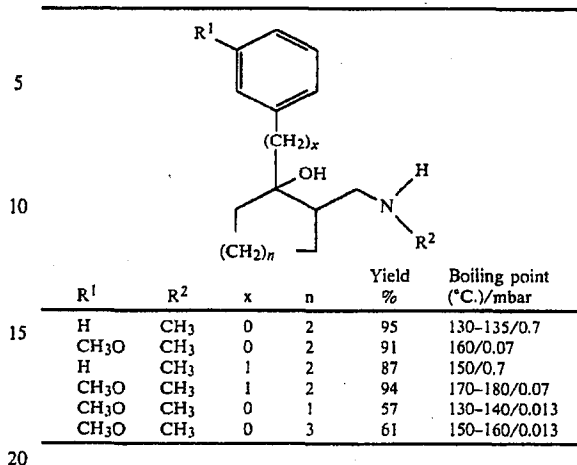
(c)  
2-(Benzylamino)-methyl-1-(3-methoxyphenyl)-cyclohexanol

43.0 g (0.103 mole) of 2-dibenzylaminomethyl-1-(3-methoxy-phenyl)-cyclohexanol (prepared as described in (b)) are dissolved in 300 ml of methanol and hydrogenated under atmospheric pressure at room temperature in the presence of 10 g of a 5% strength palladium/charcoal catalyst. When one equivalent of hydrogen has been absorbed, the hydrogenation stops. The catalyst is filtered off, the solution is concentrated and the product is distilled under reduced pressure.

Yield: 25 g (73% of theory). Boiling point 180°-190° C./0.07 mbar.

The following compounds were obtained similarly:

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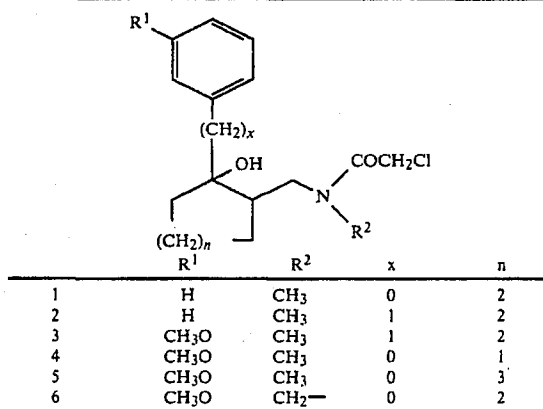


(d)  
2-(N-Chloroacetyl)-methylaminomethyl-1-(3-methoxyphenyl)-cyclohexanol

100 ml of 2 N sodium hydroxide solution are added to a solution of 30 g (0.12 mole) of 2-methylaminomethyl-1-(3-methoxyphenyl)-cyclohexanol (cf. (c)) in 300 ml of ether and 17 g (0.15 mole) of chloroacetyl chloride are then added dropwise in the course of 15 minutes, whilst stirring. Thereafter the mixture is heated for 30 minutes and then cooled, the ether layer is separated off and dried with sodium sulfate, and the solvent is distilled off. The residue is used further in its crude form.

Yield: about 100% of theory.

The following compounds were obtained similarly:



(e)  
4-Methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine-3-OH

16 g (0.05 mole) of the 2-(N-chloroacetyl)-methylaminomethyl-1-(3-methoxyphenyl)-cyclohexanol described in (c) are dissolved in 200 ml of dimethylsulfoxide, with stirring, and 10 g of potassium tert-butanolate are added a little at a time in the course of 30 minutes, whilst cooling slightly to keep the mixture at 20° C. The mixture is then heated at 50° C. for 30 minutes, after which stirring is continued overnight at room temperature. For working up, the dimethylsulfoxide is

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distilled off under reduced pressure, the residue is taken up in water, the mixture is extracted with methylene chloride and the organic phase is separated off, dried and concentrated by evaporation.

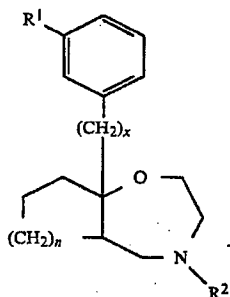
The residue is recrystallized from 2 parts of isopropanol.

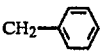
Yield: 8.5 g (65% of theory).

Melting point 120°–121° C.

Boiling point 195°–200° C./0.07 mbar.

The compounds shown below were prepared similarly, but none of them was obtainable in a crystalline form.



	R <sup>1</sup>	R <sup>2</sup>	x	n	crude yield
1	H	CH <sub>3</sub>	0	2	100
2	H	CH <sub>3</sub>	1	2	81
3	CH <sub>3</sub> O	CH <sub>3</sub>	1	2	100
4	CH <sub>3</sub> O	CH <sub>3</sub>	0	1	65
5	CH <sub>3</sub> O	CH <sub>3</sub>	0	3	100
6	CH <sub>3</sub> O	CH <sub>2</sub> - 	0	2	100

#### Preparation of the End Product

##### EXAMPLE 1

#### 4-Methyl-9a-(3-methoxybenzyl)-perhydro-1,4-benzoxazepine

40.8 g (0.14 mole) of crude 4-methyl-9a-(3-methoxybenzyl)-perhydro-1,4-benzoxazepine-3-OH (cf. (e)) are dissolved in 100 ml of absolute tetrahydrofuran and the solution is slowly added dropwise to a refluxing suspension of 9.5 g (0.25 mole) of lithium aluminum hydride in 250 ml of tetrahydrofuran. The solution is then boiled under nitrogen for 6 hours and cooled, a small amount of water is added, and after distilling off the solvent the crude base is obtained; the latter is then distilled.

The pure base is converted to its hydrochloride by means of a solution of hydrochloric acid in isopropanol.

Yield (base) 24.5 g (60% of theory).

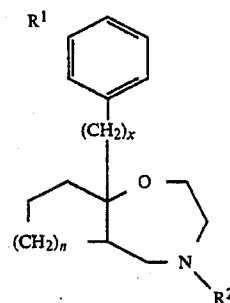
Boiling point 190°–210° C./0.27 mbar.

Hydrochloride: 220°–221° C.

The compounds tabulated below were obtained similarly:

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Example	R <sup>1</sup>	R <sup>2</sup>	x	n	Yield %	Hydrochloride Melting point, °C.
2	H	CH <sub>3</sub>	0	2	67	250
3	CH <sub>3</sub> O	CH <sub>3</sub>	0	2	74	214
4	H	CH <sub>3</sub>	1	2	58	251
5	CH <sub>3</sub> O	CH <sub>3</sub>	0	3	55	208–10
6	CH <sub>3</sub> O	CH <sub>3</sub>	0	1	46	218–20

#### EXAMPLE 7

(α)

#### 4-Methyl-9a-(3-hydroxyphenyl)-perhydro-1,4-benzoxazepine

A solution of sodium methylmercaptide in ethanol is prepared from 2.3 g (0.1 mole) of sodium, 100 ml of absolute ethanol and 6.2 g (0.1 mole) of ethylmercaptan, the alcohol is then distilled off under reduced pressure, 50 ml of dry dimethylformamide and 5.5 g (0.02 mole) of 4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine (obtained as described in Example 1) are added, and the mixture is heated for three hours at 140° C. It is then diluted with 500 ml of water and neutralized with acetic acid, after which the solution is repeatedly extracted with methylene chloride. After combining the methylene chloride extracts and removing the solvent therefrom, the residue is taken up in 100 ml of ether and the product is precipitated as the hydrochloride by introducing hydrogen chloride gas. The hydrochloride is recrystallized from isopropanol.

Yield: 4.6 g (77% of theory).

Melting point 230° C.

(β)

#### 4-Methyl-9a-(3-acetoxyphenyl)-perhydro-1,4-benzoxazepine

3.0 g (0.1 mole) of the compound obtained above and 50 ml of acetic anhydride are refluxed for 3 hours. The excess acetic anhydride is then distilled off under reduced pressure and the residue is recrystallized from a small amount of isopropanol. The compound is obtained as the hydrochloride.

Yield: 2.8 g (82% of theory).

Melting point 210° C.

#### EXAMPLE 8

#### (α) 9a-(3-Methoxyphenyl)-perhydro-1,4-benzoxazepine

16 g (0.045 mole) of 4-benzyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine (boiling point 240°–260° C./0.07 mbar), prepared by methods similar to that of Example 1, are hydrogenated in the conventional manner with a palladium/charcoal catalyst (5 g of 5% strength Pd on charcoal) in glacial acetic acid; after

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filtration and distillation, 8.8 g (75% of theory) of 9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine, of boiling point 180°-185° C./0.07 mbar, are obtained.

(β)

4-Allyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine

The compound obtained above is mixed with 80 ml of methyl isobutyl ketone, 3.2 g of allyl bromide and 3.6 g of potassium carbonate powder and the mixture is refluxed for 6 hours whilst stirring. 100 ml of water are then added, the organic layer is separated off, the solvent is removed, the residue is taken up in ether and the compound is precipitated as the hydrochloride by introducing gaseous hydrogen chloride.

After recrystallization from isopropanol, 6.4 g (57% of theory) of the hydrochloride, of melting point 225° C., are obtained.

If a similar process is carried out using ethyl bromide, 4-ethyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine is obtained.

Yield: 52% of theory.

Melting point 195°-196° C.

## EXAMPLE 9

(+) and

(-)-4-Methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine

The two optical antipodes of 4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine are prepared from (+)- and (-)-2-(N-benzyl-methylamino)-methyl-1-(3-methoxyphenyl)-cyclohexanol, obtained as described in (b), by employing processes c, d and e and the process of Example 1.

Specific optical rotations (measured in methanol, C=20 mg/ml):

Bases:  $[\alpha]_D^{20} = \pm 15^\circ$ .

Hydrochlorides:  $[\alpha]_D^{20} = \pm 35^\circ$ .

Melting points: 190°-191° C.

## EXAMPLE 10

Tablets of the following composition are molded on a tableting press in the conventional manner:

20.00 mg of 4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine

50.00 mg of corn starch

4.50 mg of gelatin

15.00 mg of lactose

7.50 mg of talc

0.75 mg of Aerosil® (chemically pure silica in a sub-microscopic state of fine division)

2.25 mg of potato starch (as a 6% strength paste).

## EXAMPLE 11

Dragees of the following composition are prepared in the conventional manner:

20.00 mg of 4-methyl-9a-(3-acetoxyphenyl)-perhydro-1,4-benzoxazepine

50.00 mg of core composition

40.00 mg of sugar-coating composition.

The core composition consists of 9 parts of corn starch, 3 parts of lactose and 1 part of Luviskol® VA 64 (a 60:40 vinylpyrrolidone/vinyl acetate copolymer, cf. Pharm. Ind. 1962, 586). The sugar-coating composition consists of 5 parts of cane sugar, 2 parts of corn starch, 2 parts of calcium carbonate and 1 part of talc. The dragees thus prepared are subsequently provided with a coating which is resistant to gastric juices.

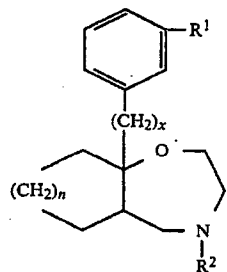
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## EXAMPLE 12

10 g of 4-methyl-9a-(3-methoxybenzyl)-perhydro-1,4-benzoxazepine hydrochloride are dissolved in 2.0 liters of water and the solution is made isotonic acid sodium chloride and is filled, under sterile conditions, into ampoules of 2 ml capacity.

We claim:

1. A 1,4-Cycloalkano-oxazepine of the general formula I



where R<sup>1</sup> is hydrogen, hydroxyl or alkoxy or acyloxy of 1 to 4 carbon atoms, R<sup>2</sup> is a hydrocarbon radical of 1 to 3 carbon atoms, n is 1, 2 or 3 and x is 0 or 1, or one of its salts with physiologically acceptable acids.

2. A compound selected from the group consisting of 4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine, (+)-4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine, (-)-4-methyl-9a-(3-methoxyphenyl)-perhydro-1,4-benzoxazepine, 4-methyl-8a-(3-methoxyphenyl)-perhydro-1,4-cyclopentoxazepine, 4-methyl-9a-(3-hydroxyphenyl)-perhydro-1,4-benzoxazepine and 4-methyl-9a-(3-acetoxyphenyl)-perhydro-1,4-benzoxazepine.

3. A pharmaceutical composition comprising a galenic auxiliary containing an analgesically effective dose of a compound as claimed in claim 2.

4. A pharmaceutical composition comprising a galenic auxiliary containing an analgesically effective dose of a compound as claimed in claim 1.

5. A therapeutic composition comprising a liquid or solid suitable for oral or parenteral administration, said composition containing an analgesically effective amount of a compound as claimed in claim 2.

6. A therapeutic composition comprising a liquid or solid suitable for oral or parenteral administration, said composition containing an analgesically effective amount of a compound as claimed in claim 1.

7. A cycloalkano-oxazepine as claimed in claim 1 wherein the compound is one of said salts.

8. A cycloalkano-oxazepine as claimed in claim 13 wherein said salt is a salt of one of hydrochloric acid, sulfuric acid, phosphoric acid, tartaric acid, citric acid, fumaric acid, acetic acid, formic acid, succinic acid, maleic acid, lactic acid, and amidosulfonic acid.

9. A salt of one of the acids hydrochloric acid, sulfuric acid, phosphoric acid, tartaric acid, citric acid, fumaric acid, acetic acid, formic acid, succinic acid, maleic acid, lactic acid, and amidosulfonic acid and a compound as claimed in claim 2.

10. Process for alleviating pain in patients suffering therefrom, characterized in that an effective quantity of a compound according to claim 1 is administered to the patients.

11. Process for alleviating pain in patients suffering therefrom, characterized in that an effective quantity of a compound according to claim 2 is administered to the patients.

\* \* \* \* \*

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# EXHIBIT 23

**United States Patent** [19]

Schorr et al.

[11] **4,018,933**[45] **Apr. 19, 1977****[54] ACYLAMINOPENICILLANIC ACIDS AND  
PROCESS FOR PREPARING THEM**

[75] Inventors: **Manfred Schorr**, Frankfurt am Main;  
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**Wilfried Schmitt**, Kelkheim, Taunus,  
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[73] Assignee: **Hoechst Aktiengesellschaft**,  
Frankfurt am Main, Germany

[22] Filed: **Dec. 11, 1975**

[21] Appl. No.: **639,807**

**[30] Foreign Application Priority Data**

Dec. 13, 1974 Germany ..... 2458973

[52] U.S. Cl. .... **424/271; 260/239.1**

[51] Int. Cl.<sup>2</sup> ..... **C07D 499/68; C07D 499/70;**  
A61K 31/43

[58] Field of Search ..... 260/239.1; 424/271

**[56] References Cited****UNITED STATES PATENTS**

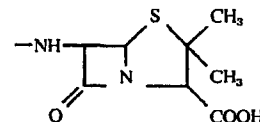
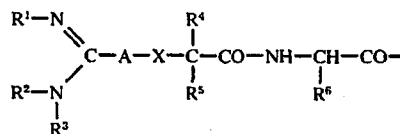
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*Primary Examiner*—Gerald A. Schwartz

*Attorney, Agent, or Firm*—Curtis, Morris & Safford

**[57] ABSTRACT**

Acylaminopenicillanic acids of the general formula



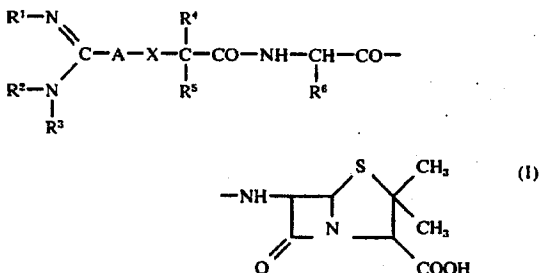
in which R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> represent hydrogen or lower alkyl which may be substituted, and in which the radicals R<sup>1</sup> and R<sup>2</sup> or R<sup>2</sup> and R<sup>3</sup> may form together an alkylene radical which may be substituted, R<sup>4</sup> and R<sup>5</sup> represent hydrogen or lower alkyl, R<sup>6</sup> represents phenyl which may be substituted, a monocyclic aromatic heterocycle which may be substituted, or dihydrophenyl, A represents a benzene or thiophene ring which may be substituted, and X represents oxygen or a single bond, a process for preparing them and pharmaceutical compositions active against bacterial infections containing these compounds.

**16 Claims, No Drawings**

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# ACYLAMINOPENICILLANIC ACIDS AND PROCESS FOR PREPARING THEM

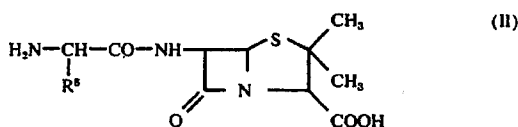
The present invention provides novel penicillins of the general formula I



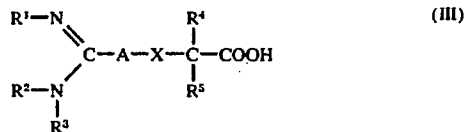
in which  $\text{R}^1$ ,  $\text{R}^2$  and  $\text{R}^3$  represent hydrogen or lower alkyl which may be substituted, and in which the radicals  $\text{R}^1$  and  $\text{R}^2$  or  $\text{R}^2$  and  $\text{R}^3$  each together may form an alkylene radical which may be substituted,  $\text{R}^4$   $\text{R}^5$  represent hydrogen or lower alkyl,  $\text{R}^6$  represents phenyl which may be substituted, a monocyclic aromatic heterocycle which may be substituted or dihydrophenyl, A represents a benzene or thiophene ring which may be substituted, and X represents oxygen or a single bond.

Furthermore, the invention relates to a process for preparing penicillins of the general formula I, in which  $\text{R}^1$  to  $\text{R}^6$ , A and X have the meanings given above, which comprises

a. reacting aminopenicillins of the general formula II

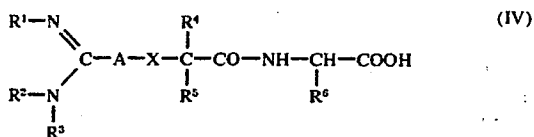


in which  $\text{R}^6$  has the meaning given above, or salts or derivatives thereof which are protected at the carboxyl group, with carboxylic acids of the general formula III



in which  $\text{R}^1$  to  $\text{R}^5$ , A and X have the meanings given above, or with reactive derivatives of such acids, or

b. reacting 6-aminopenicillanic acid or a salt or a derivative thereof which is protected at the carboxyl group, with an acid of the general formula IV



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in which  $\text{R}^1$  to  $\text{R}^6$ , A and X have the meanings given above, or with a reactive derivative of such an acid, and splitting off any protecting groups present.

If  $\text{R}^1$ ,  $\text{R}^2$  and  $\text{R}^3$  represent alkyl radicals, these may be straight chain or branched alkyl radicals of 1 to 5 carbon atoms and the sum of the carbon atoms in the three radicals should not be higher than 8. Possible substituents of the alkyl radicals are, preferably, low molecular weight alkoxy groups, for example methoxy or ethoxy.

If  $\text{R}^1$  and  $\text{R}^2$  or  $\text{R}^2$  and  $\text{R}^3$  together form an alkylene radical, the latter preferably contains 2 to 4 carbon atoms. It may also be interrupted by a hetero-atom, preferably oxygen or nitrogen. As substituents of the alkylene radical, there may be used in particular low molecular alkyl radicals containing 1 to 4 carbon atoms and optionally also a hetero-atom, preferably oxygen. These alkyl radicals themselves may also be closed to a ring which may be interrupted by a hetero-atom, preferably an oxygen atom.

If  $\text{R}^4$  and  $\text{R}^5$  are low molecular alkyl radicals, these may contain preferably 1 to 3 carbon atoms.

$\text{R}^6$  may represent in particular phenyl, but also substituted phenyl, the substituents being, for example hydroxy, preferably in the 4-position, alkyl of 1 to 4 carbon atoms, preferably methyl, alkoxy of 1 to 4 carbon atoms, preferably methoxy, or halogen, preferably chlorine or fluorine.  $\text{R}^6$  may furthermore represent dihydrophenyl such as 2,5-dihydrophenyl, or a monocyclic aromatic heterocycle, for example 2- or 3-thienyl, 2- or 3-furyl or 2- or 3-pyridyl, in which the heterocycles may also be further substituted, for example by alkyl of 1 to 4 carbon atoms, preferably methyl, or lower alkoxy, preferably methoxy.

A may represent a benzene ring or thiophene ring which may be substituted, in particular a 1,4-phenylene- or 2,5-thienylene radical. The substituents may be, for example low molecular alkoxy of 1 to 4 carbon atoms, preferably methoxy, halogen, preferably fluorine or chlorine, or low molecular alkyl, preferably methyl.

Penicillins which fall within the scope of the invention are, for example:

6-[D,L-2-(4-amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid,  
6-[D-2-(4-Amidinophenoxyacetyl-amino)-2-P +  
6-[2-( $\alpha$ -4-Amidinophenoxy>-propionyl-amino)-2-P +  
6-[2-( $\alpha$ -4-Amidinophenoxy>-butyryl-amino)-2-P +  
6-[2-( $\alpha$ -4-Amidinophenoxy>-isobutyryl-amino)-2-P +  
6-[2-( $\alpha$ -4-Amidinophenoxy>-valeryl-amino)-2-P +  
6-[2-(4-Amidino-2-chlorphenoxyacetyl-amino)-2-P +  
6-[2-(4-Amidino-2-methylphenoxyacetyl-amino)-2-P +  
6-[2-(4-Amidino-2-methoxyphenoxyacetyl-amino)-2-P +  
6-[2-(4-Amidino-3-chlorphenoxyacetyl-amino)-2-P +  
6-[2-(3-Amidinophenoxyacetyl-amino)-2-P +  
6-[2-(4-Amidinophenylacetyl-amino)-2-P +  
6-[2-(4-N-Methylamidinophenylacetyl-amino)-2-P +  
6-[2-(4-N,N-Dimethylamidinophenylacetyl-amino)-2-P +  
6-[2-(4-N,N'-Dimethylamidinophenylacetyl-amino)-2-P +  
6-[2-(4-N,N,N'-Trimethylamidino-phenylacetyl-amino)-2-P +  
6-[2-(4-N-Ethylamidinophenylacetyl-amino)-2-P +  
6-[2-(4-N,N-Diethylamidinophenylacetyl-amino)-2-P +  
6-[2-(4-N-Propylamidinophenylacetyl-amino)-2-P +

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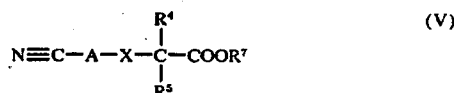
6-[2-(4-N,N'-Dipropylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N-Isopropylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N,N'-Diisopropylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N-Butylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N,N-Dibutylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N-Pentylamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N-Methyl-N-propylamidino-phenylacetyl-amino)-2-P +  
 6-[2-(4-N,N-Dimethyl-N'-ethylamidono-phenylacetyl-amino)-2-P +  
 6-[2-(2-N,N-Trimethylenamidinophenylacetyl-amino)-2-P +  
 6-[2-(4-N,N-Tetramethylenamidono-phenylacetyl-amino)-2-P +  
 6-[2-(4-N,N-Pentamethylenamidino-phenylacetyl-amino)-2-P +  
 6-[2-(4-<3-Azabicyclo[3,3,1]nonan-3-yl-carbonimidoyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-Morpholinocarbonimidoyl-phenylacetyl-amino)-2-P +  
 6-[2-(4-<4-Methylpiperazin-1-yl-carbonimidoyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<2-Imidazolyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<1,5-Dimethyl-2-imidazolyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<1,4,5,6-Tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<1-Methyl-1,4,5,6-tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<1-Ethyl-1,4,5,6-tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<5,5-Dimethyl-1,4,5,6-tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<5,5-Diethyl-1,4,5,6-tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<5,5-Dipropyl-1,4,5,6-tetrahydro-2-pyrimidyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<5,5-Bis-methoxyethyl-1,4,5,6-tetrahydro-2-pyriminyl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-N,N'-Tetramethylenamidino-phenylacetyl-amino)-2-P +  
 6-[2-(4-<1,4,6,7,8,9-Hexahydro-5H-cyclopenta[d]-pyrimid-2-yl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<2,4-Diazospiro[5,5]undec-2-en-3-yl>-phenylacetyl-amino)-2-P +  
 6-[2-(4-<9-Oxa-diazospiro[5,5]undec-2-en-3-yl>-phenylacetyl-amino)-2-P +  
 6-[2-(5-Amidinothien-2-ylacetyl-amino)-2-P +  
 P + represents "phenylacetyl-amino]-penicillanic acid

In each of the above compounds, P may also represent, for example:

2-hydroxyphenylacetyl-amino]-penicillanic acid  
 3-hydroxyphenylacetyl-amino]-penicillanic acid  
 4-hydroxyphenylacetyl-amino]-penicillanic acid  
 3,5-dihydroxyphenylacetyl-amino]-penicillanic acid  
 2-methylphenylacetyl-amino]-penicillanic acid  
 3-methylphenylacetyl-amino]-penicillanic acid  
 4-methylphenylacetyl-amino]-penicillanic acid  
 2-methoxyphenylacetyl-amino]-penicillanic acid  
 3-methoxyphenylacetyl-amino]-penicillanic acid  
 4-methoxyphenylacetyl-amino]-penicillanic acid  
 2-chlorophenylacetyl-amino]-penicillanic acid  
 3-chlorophenylacetyl-amino]-penicillanic acid  
 4-chlorophenylacetyl-amino]-penicillanic acid  
 2-fluorophenylacetyl-amino]-penicillanic acid

3-fluorophenylacetyl-amino]-penicillanic acid  
 4-fluorophenylacetyl-amino]-penicillanic acid  
 2,5-dihydroxyphenylacetyl-amino]-penicillanic acid  
 2-thienylacetyl-amino]-penicillanic acid  
 3-thienylacetyl-amino]-penicillanic acid  
 3-methyl-2-thienylacetyl-amino]-penicillanic acid  
 2-furylacetyl-amino]-penicillanic acid  
 3-furylacetyl-amino]-penicillanic acid  
 2-pyridylacetyl-amino]-penicillanic acid  
 3-pyridylacetyl-amino]-penicillanic acid  
 4-pyridylacetyl-amino]-penicillanic acid  
 The acids of the general formula III may be obtained in known manner from cyano compounds of the general formula V

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in which R<sup>4</sup>, R<sup>5</sup>, A and X have the meanings given above and R<sup>7</sup> represents low molecular alkyl. After conversion of the nitrile group into an imino-ether, the latter is reacted with ammonia or an amine or a diamine to the amidine and finally the carbon-ester group or an acid group resulting from it in the course of the reaction is saponified. Aminopenicillins of the formula II can be prepared, for example according to the process described in German Auslegeschrift 1 139 844.

The novel penicillins of the general formula I can be prepared by reaction of a carboxylic acid of the general formula II with an aminopenicillin of the general formula II, for example in known manner in the presence of a carbodiimide such as dicyclohexylcarbodiimide as condensation agent.

The novel penicillins of the general formula I are obtained in a particularly advantageous manner by reacting the acid of the general formula III in the form of a reactive derivative with an aminopenicillin of the general formula II. The acid chlorides have especially well proved. They may be obtained from the carboxylic acids in known manner by the action of thionyl chloride. An excess of thionyl chloride may simultaneously serve as solvent. In some cases, however, it is of advantage to carry out the reaction in the presence of an inert solvent or diluent, for example an aromatic hydrocarbon. The acid chlorides are obtained in the form of hydrochlorides which may be used directly for the further reaction. Besides the acid chlorides, also other derivatives of the carboxylic acids of the formula III may be used, for example acid bromides, activated esters, for example the p-nitrophenyl ester, the p-nitrophenylthio ester or the cyanomethyl ester, acid azides or symmetrical or mixed anhydrides.

The aminopenicillins of the general formula II are advantageously acylated in the form of their salts. Suitable salts are, for example alkali metal salts or tertiary salts such as the sodium, potassium or triethylamino salt. These salts may be used directly in the reaction or prepared in the reaction mixture from the aminopenicillin and suitable bases such as sodium hydrogencarbonate, di-sodium-hydrogenophosphate or triethylamine. The acylation itself is effected in general in the presence of a solvent or diluent. Suitable for this purpose is, for example water in which the salt of the aminopenicillin of the formula II is dissolved or dispersed. It has particularly well proven to introduce the

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hydrochloride of the acid chloride in solid form. In order to bind the forming hydrogen chloride, another mole of a base such as sodiumhydrogenocarbonate or triethylamine is added. The reaction is carried out at room temperature or at slightly reduced temperatures, preferably between about  $-5^{\circ}$  and  $+5^{\circ}$  C. In general, the penicillin of the formula I precipitates in sparingly soluble form and can be isolated by filtration.

The acylation of the aminopenicillins of the formula II may also be effected in the presence of organic solvents. Solvents of the type of dimethylformamide and dimethylsulfoxide or halogenated hydrocarbons such as methylene chloride or chloroform have well provided. Dimethylformamide, in which the triethylamine salts of the aminopenicillins dissolve, is used in particularly advantageous manner. The hydrochlorides of the acid chlorides of the carboxylic acids of the formula III may be introduced in solid form into this solution, while maintaining the whole preferably at room temperature or at slightly reduced temperatures. When dimethylformamide is used as solvent, the novel penicillins that have formed generally remain dissolved. After removal of precipitated salts, they can be isolated by the addition of a suitable precipitant, for example diethyl ether or diisopropyl ether.

The reactive derivatives of the carboxylic acids of the general formula III may not be reacted with the aminopenicillins of the general formula II, but also with derivatives of these compounds. In this respect, there may be used above all the esters which may be split optionally in a neutral, acidic or weakly basic medium by solvolysis, for example by hydrolysis or alcoholysis, hydrogenolysis, by reduction, by nucleophilic exchange or photolysis, to the free carboxyl group.

Ester groups which are easily split by solvolysis with a solvent containing hydroxy groups, for example water or alcohols, preferably under neutral conditions, are those which are derived from phosphinyl-, silyl-, germanyl-, plumbyl- or stannyl-alcohols, for example those described in DOS 2 222 094 (1972), British Pat. No. 1,073,530, Netherland Patent Publication No. 67/17107 or DOS 1 800 698. Preferred are the groups of the general formula  $R^7R^8P(O)-O-CO-$  or  $R^7R^8R^9Si-O-CO-$ , in which  $R^7$ ,  $R^8$ ,  $R^9$  may be identical or different and represent preferably lower alkyl or aryl, for example phenyl.

Esters which are easily split in an acid medium are those which are derived from lower alcohols which are polybranched in the  $\alpha$ -position or contain one or several electron donors and optionally substituted aromatic hydrocarbons or heterocycles of aromatic nature or aroyl radicals or acyloxy radicals. As Examples thereof, there may be mentioned the tert.butyl ester, the cyclohexyl ester, the adamantyl ester, the 2-tetrahydropyranyl ester, the p-nitrobenzyl ester, the benzhydryl ester, the trityl ester, the 3,4-dimethoxybenzyl ester, the benzoylmethyl ester, the acetoxymethyl ester or the pivaloyloxymethyl ester.

Ester groups which can be split by hydrolysis in a weakly basic or acidic medium are, for example activated esters which are derived from an optionally substituted phenol or benzylalcohol, for example the 4-nitrophenyl-, 2,4-dinitrophenyl-, 4-nitrobenzyl- or triphenylmethyl ester.

The esters which are derived, for example from an optionally substituted benzyl alcohol, for example the 4-nitrobenzyl alcohol, may also be split by hydrogenolysis.

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Ester groups which are derived from halogenated low molecular alcohols, for example the 2,2,2-trichloroethanol, the 2-chloroethanol, the 2-bromoethanol or the 4-pyridylmethanol, may be split reductively by treatment with nascent hydrogen or by electrolytic reduction.

Ester groups which can be split by photolysis, for example by ultraviolet light, are those derived from methanols which may be substituted by aryl. Such groups are, for example 4-methoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl or 2-nitrobenzyloxycarbonyl.

The reaction of these derivatives of the aminopenicillins of the general formula II with reactive derivatives of the carboxylic acids of the general formula III may be carried out in the presence of inert solvents or diluents in the manner indicated for the use of the salts of the formula II. It is followed by a splitting reaction, effected in known manner, for example by solvolysis, for example saponification by the action of water or diluted acids, or a reductive splitting, for example by catalytically excited hydrogen, or a photolytic splitting, for example by irradiation with ultraviolet light under neutral or acidic conditions.

The novel penicillins of the general formula I may also be obtained by reacting acids of the general formula IV with 6-aminopenicillanic acid. This can be effected, for example in known manner in the presence of a carbodiimide such as dicyclohexylcarbodiimide as condensing agent. It is particularly advantageous to use the acids of the formula IV in the form of their reactive derivatives. In this case too in particular the acid chlorides in the form of hydrochlorides have proved satisfactory. Otherwise, the reaction is carried out in a manner analogous to that described for the reaction of the acids of the general formula III with the aminopenicillins of the general formula II.

The 6-aminopenicillanic acids may also be used in the form of easily splittable derivatives. As such, there may be used in particular such esters as those already mentioned in the case of the aminopenicillins of the general formula II. If they are esters which can be easily split by hydrolysis, the reaction with the acids of the general formula IV or the derivatives thereof is carried out with the exclusion of water. Otherwise the reaction may be carried out as described for the reaction of the compounds II and III.

The acids of the general formula IV used as starting product may be obtained from the corresponding aminoacetic acids or their esters by the action of reactive derivatives of the acids of the general formula III and optional subsequent saponification of the ester groups present.

The novel penicillins of the general formula I have amphoteric character and constitute inner salts. In general, they are colourless crystalline compounds which have a different, easy solubility in water.

They have interesting antibiotic properties with a broad activity spectrum against bacterial germs. The action against gram-negative problem germs, for example Pseudomans or Proteus is particularly marked. By reason of these properties, the novel compounds represent valuable therapeutics.

The minimum inhibition concentrations of 6-[D-2-(4-<1,4,5,6-tetrahydropyrimide-2-yl>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid (compound "A", obtained according to Example 12) and Carbenicillin were determined on a series of

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strains. The tests were carried out according to the series dilution test with Antibiotic Medium III of Difco.

Germs	Minimum inhibition concentration, in weight units/ml	
	A	Carbenicillin
Strept. Aronson B	0.125 mcg	0.625 mcg
agalactiae B	0.195 mcg	1.25 mcg
faecium	7.813 mcg	62.5 mcg
faecalis D	31.25 mcg	250.0 mcg
Pseud. aeruginosa 1592E	31.25 mcg	125.0 mcg
1593E	31.25 mcg	500.0 mcg
1594E	31.25 mcg	250.0 mcg

The compounds of the invention may be used as such or together with the therapeutically usual adjuvants and excipients, for example tragacanth, lactose, talc, solvents and the like, in the form of galenical preparations, for example tablets, dragees, capsules, suspensions or solutions, they may be administered perorally or, preferably, parenterally, the active substance being contained in a dosage unit, in general in a quantity of about 50 to 1000 mg, preferably about 100 to 500 mg.

For parental administration, there is preferably used a solution in water which is suitably prepared shortly before administration.

It is also possible to combine the compounds of the invention with other active substances. Thus, they may be combined and administered with other antibiotics, for example those of the series of penicillins, cephalosporins, or with compounds which have an influence on the symptomatic of bacterial infections, for example antipyretics, antiphlogistics or analgesics.

The following Examples illustrate the invention.

#### EXAMPLES

The novel penicillins described in the following Examples are characterized by the  $R_f$ -value of the thin layer chromatogram. As the layer, silica gel (Merck) and as solvent, a mixture of n-butanol-glacial acetic acid-water in a ratio of 6:2:2 were used. Development of the plates was effected by the action of iodine vapour.

#### EXAMPLE 1

##### 6-[D-2-(4-Amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 19.4 g of 4-amidinophenoxyacetic acid and 59.5 g of thionyl chloride were well stirred for 4 hours, at a bath temperature of 65° C, in 100 ml of benzene, after addition of 2 drops of dimethylformamide. After cooling, the whole was filtered with suction, washed with benzene and diisopropyl ether and the product was dried under reduced pressure at room temperature. Yield: 23.2 g, F.p. 145° to 147° C (decomposition).

b. 5 g of 4-amidinophenoxyacetic acid chloride hydrochloride were introduced at 0° C into a solution of 7 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 4.46 g of triethylamine in 100 ml of anhydrous dimethylformamide. The reaction mixture was stirred for 30 minutes at 0° C and for 1 hour at room temperature. After removal of the precipitated triethylamine-hydrochloride by suction-filtration, the filtrate was slowly combined with 400 ml of diethyl ether. The powdery product which had precipitated was filtered off with suction and, in order to eliminate

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any triethylamino hydrochloride and 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid still present, it was distributed in a mixture of 500 ml of methylene chloride and 5 ml of triethylamine and stirred for 45 minutes. The product was filtered off with suction and washed with methylene chloride and diethyl ether. Yield: 7.55 g; decomposition point 198°-200° C.  $R_f$ -value = 0.52.

#### EXAMPLE 2

##### 6-[D-2-(4-amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

8.06 of 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid-trihydrate and 10.08 g of sodium bicarbonate were dispersed in 100 ml of water at 0° C. 5.0 g of 4-amidinophenoxyacetic acid chloride hydrochloride were introduced, while well stirring, and the whole was stirred for 30 minutes at 0° C. The product was then filtered off with suction, washed with ice water and dried under reduced pressure. 7.8 g of a raw product were obtained which were then treated as described in Example 1(b) with methylene chloride and triethylamine. 6.6 g of colourless crystals were obtained. In order to eliminate small amounts of 4-amidinophenoxy-acetic acid, the product was treated with 100 ml of dimethylformamide and undissolved matter was filtered off with suction. From the filtrate, the 6-[D-2-(4-amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid was precipitated by the addition of diethyl ether. Yield: 2 g; decomposition point 198° to 200° C;  $R_f$ -value = 0.52.

#### EXAMPLE 3

##### 6-[D-2-(4-amidinophenoxyacetyl-amino)-2-(4-hydroxyphenyl)-acetyl-amino]-penicillanic acid

4.5 g of anhydrous 6-(D- $\alpha$ -amino-4-hydroxyphenylacetyl-amino)-penicillanic acid and 2.52 g of triethylamine were dissolved at 0° C in 60 ml of anhydrous dimethylformamide and, at the same temperature, 3.05 g of 4-amidinophenoxyacetic acid chloride hydrochloride were introduced. The reaction mixture was then stirred for 90 minutes at 0° C and the triethylaminehydrochloride was filtered off with suction. Upon addition of diethyl ether, the above-mentioned penicillin precipitated from the filtrate. This raw product was treated as described in Example 1(b) with methylene chloride and triethylamine. After suction-filtration, washing with methylene chloride and diethyl ether and drying under pressure reduced pressure, the yield was 4.7 g. Decomposition point 192° to 194° C;  $R_f$ -value = 0.50.

#### EXAMPLE 4

##### 6-[D-2-(3-amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 19.4 g of 3-amidinophenoxyacetic acid were heated under reflux, while well stirring, for 2 hours with 70 ml of thionyl chloride. After cooling, filtration with suction and washing with anhydrous diethyl ether, 21 g of 3-amidinophenoxyacetic acid chloride hydrochloride were obtained; decomposition point 149° to 151° C.

b. 7 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 4.46 g of triethylamine were dissolved at 0° C in 100 ml of anhydrous dimethylformamide and then, 5.0 g of 3-amidino-

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phenoxyacetic acid chloride-hydrochloride were added portionwise. The reaction mixture was stirred for 30 minutes at 0° C and for 1 hour at room temperature. Working up was effected as described in Example 1(b). Yield: 5.6 g; decomposition point 196° to 198° C;  $R_f$ -value = 0.53.

## EXAMPLE 5

## 6-[D-2-(4-amidinophenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. The 4-amidinophenylacetic acid chloride-hydrochloride was obtained from 4-amidinophenylacetic acid as described in Example 1(a). Decomposition point: 174° to 177° C.

b. Starting from 8.75 g of 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid (anhydrous) and 5.82 g of 4-amidino phenylacetic acid chloride-hydrochloride, there were obtained in a manner analogous to the method described in Example 1(b), 10.5 g of the above-mentioned compound. Decomposition point: 190° to 193° C;  $R_f$ -value = 0.46.

## EXAMPLE 6

## 6-[D-2-(4-amidino-2-methoxyphenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. According to the method described in Example 1(a), there was prepared the 4-amidino-2-methoxyphenoxyacetic acid chloride-hydrochloride from 6.8 g of 4-amidino-2-methoxy-phenoxyacetic acid and a mixture of 30 ml of benzene and 30 ml of thionyl chloride. Decomposition point: 153° to 156° C.

b. By the reaction of 8.75 g of 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid (anhydrous) with 7 g of 4-amidino-2-methoxyphenoxyacetic acid chloride-hydrochloride according to the method described in Example 1(b), there were obtained 11 g of the above-mentioned penicillin. Decomposition point 189° to 191° C;  $R_f$ -value = 0.45.

## EXAMPLE 7

## 6-[D-2-(4-amidinophenoxyisobutyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 25 ml of thionyl chloride was poured over 4.44 g of 4-amidinophenoxyisobutyric acid and the whole was stirred for 2 hours at room temperature. After some minutes, a clear solution was obtained. Then, 100 ml of anhydrous diethyl ether were introduced, while stirring and the crystallizing 4-amidinophenoxyisobutyric acid chloride-hydrochloride was filtered off with suction and washed with anhydrous ether. Yield: 5 g; decomposition point 160° to 161° C.

b. By working as described in Example 1(b), there were obtained from 6.3 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 5.0 g of 4-amidino-phenoxyisobutyric acid chloride-hydrochloride, 7.2 g of the above-mentioned penicillin. Decomposition point: 191°;  $R_f$ -value = 0.57.

## EXAMPLE 8

## 6-[D-2-(4-(2-imidazolyl)-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 5.8 g of 4-(2-imidazolyl)-phenoxyacetic acid were heated for 4 hours under reflux with 40 ml of benzene and 40 ml of thionyl chloride and stirred. The reaction mixture was then cooled and the 4-(2-imidazolyl)-phenoxyacetic acid chloride-hydrochloride

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ride that had crystallized was filtered off with suction and washed with anhydrous ether. Yield: 6.4 g; decomposition point from 195° C onwards.

b. Under the conditions described in Example 1(b), there were obtained from 7.0 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 5.5 g of 4-(2-imidazolyl)-phenoxyacetic acid chloride-hydrochloride, 6.5 g of the above-mentioned penicillin. Decomposition point: 200° to 202° C;  $R_f$ -value = 0.35.

## EXAMPLE 9

## 6-[D-2-(4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 5.4 g of 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid hydrochloride were combined with 40 ml of thionyl chloride and the whole was heated for 1 hour under reflux. After cooling, the reaction solution was combined slowly with 100 ml of anhydrous diethyl ether. Thereupon, the 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid chloride-hydrochloride crystallized; it was filtered off with suction and washed with anhydrous ether. Yield: 5.6 g; decomposition point 200° to 202° C.

b. The above-mentioned penicillin was obtained in a manner analogous to that described in Example 1(b) from 6.55 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 5.4 g of 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid chloride-hydrochloride. Yield: 8.4 g; decomposition point: 211° to 213° C;  $R_f$ -value = 0.37.

## EXAMPLE 10

## 6-[D-2-(4-(5,5-dimethyl-1,4,5,6-tetrahydropyrimide-2-yl)-phenoxy-acetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. A mixture of 5.24 of 4-(5,5-dimethyl-1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid and 20 ml of thionyl chloride was stirred for 1 hour at 40° C. The reaction mixture was then poured into 100 ml of anhydrous diethyl ether and the 4-(5,5-dimethyl-1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid chloride-hydrochloride that had precipitated was filtered off with suction, washed with ether and dried under reduced pressure. Yield: 5.8 g; decomposition point 185° C.

b. In a manner analogous to that described in Example 1(b), there were obtained from 6.3 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 5.8 g of 4-(5,5-dimethyl-1,4,5,6-tetrahydropyrimide-2-yl)-phenoxyacetic acid chloride-hydrochloride, 5.8 g of the above-mentioned compound. Decomposition point 195° C.  $R_f$ -value = 0.51.

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## -[D-2-(4-(9-oxa-2,4-diazaspiro[5,5]undec-2-ene-3-yl)-phenoxy-acetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 3.4 g of 4-(9-oxa-2,4-diazaspiro[5,5]undec-2-ene-3-yl)-phenoxyacetic acid hydrochloride and 15 ml of thionyl chloride were heated for about 20 minutes on the steam bath until dissolution was complete. The whole was allowed to stand for several hours at room temperature, poured into 100 ml of anhydrous diethyl ether and the 6-(9-oxa-2,4-diazaspiro[5,5]undec-

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2-ene-3-yl)-phenoxyacetic acid chloride-hydrochloride that had precipitated in crystalline form was filtered off with suction. Yield: 2.5 g; decomposition point from 198° C onwards.

b. 2.45 g of anhydrous 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid were reacted in a manner analogous to that described in Example 1(b) with 2.5 g of 4-(9-oxa-2,4-diazaspiro[5,5]undec-2-ene-3-yl)-phenoxyacetic acid chloride-hydrochloride, whereupon 3.5 g of the above mentioned penicillin were obtained. Decomposition point: 210° to 212° C;  $R_f$ -value = 0.41.

## EXAMPLE 12

6-[D-2-(4-<1,4,5,6-Tetrahydropyrimide-2-yl>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 5.08 g of 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenylacetic acid hydrochloride were reacted with 15 ml of thionyl chloride in a manner analogous to that described in Example 7(a), whereupon 5.3 g of 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenylacetic acid chloride-hydrochloride were obtained.

b. 3.5 g of anhydrous 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid were reacted with 2.73 g of 4-(1,4,5,6-tetrahydropyrimide-2-yl)-phenylacetic acid chloride-hydrochloride in a manner analogous to that described in Example 1(b), whereby 3.95 g of the above-mentioned compound were obtained. For further purification, the substance was dissolved in water, filtered and lyophilized.  $R_f$ -value = 0.41.

## EXAMPLE 13

6-[D-2-(4-<N,N-Pentamethyleneamidino>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 5.97 g of 4-(N,N-pentamethyleneamidino)-phenoxyacetic acid-hydrochloride were reacted with mixture of 25 ml of benzene and 25 ml of thionyl chloride in a manner analogous to that described in Example 8(a). 6.0 g of 4-(N,N-Pentamethyleneamidino)-phenoxyacetic acid chloride-hydrochloride were obtained; decomposition point 186° to 188° C.

b. 6.5 g of anhydrous 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid and 5.9 g of 4-(N,N-pentamethyleneamidino)-phenoxyacetic acid chloride-hydrochloride were reacted as described in Example 1(b). Yield: 5.0 g of the above-specified penicillin;  $R_f$ -value = 0.46.

## EXAMPLE 14

6-[D-2-(4-<3-Azabicyclo[3,3,1]nonane-3-yl-carbonimidoyl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 6.04 g of 4-(3-azabicyclo[3,3,1]nonane-3-yl-carbonimidoyl)-phenoxyacetic acid were reacted as described in Example 8(a) with a mixture of 25 ml of benzene and 25 ml of thionyl chloride. Yield: 7.0 g of 4-(3-azabicyclo[3,3,1]nonane-3-yl-carbonimidoyl)-phenoxyacetic acid chloride-hydrochloride.

b. 6.84 g of 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid (anhydrous) were reacted in a manner analogous to the method described in Example 1(b) with 7.0 g of 4-(3-azabicyclo[3,3,1]nonane-3-yl-carbonimidoyl)-phenoxyacetic acid chloride-hydrochloride. For further purification, the crude product ob-

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tained was treated with 30 ml of water, filtered off with suction, washed well with water and dried under reduced pressure. Yield: 8.0 g; decomposition point about 190° C;  $R_f$ -value = 0.51.

## EXAMPLE 15

6-[D-2-(4-<2-ImidazolinyI>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 10 g of 4-(2-ImidazolinyI)-phenylacetic acid were stirred for 5 hours at 55° C with a mixture of 150 ml of benzene and 90 ml of thionyl chloride. The reaction mixture was then cooled, filtered with suction, washed with benzene and diethyl ether and dried under reduced pressure. 11 g of 4-(2-ImidazolinyI)-phenylacetic acid chloride-hydrochloride were obtained.

b. 4.05 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 3.0 g of 4-(2-imidazolinyI)-phenylacetic acid chloride-hydrochloride were reacted in a manner analogous to that described in Example 1(b). 3.6 g of the above-specified penicillin were obtained. For further purification, the product was dissolved in 100 ml of water, filtered and lyophilized.  $R_f$ -value = 0.38.

## EXAMPLE 16

6-[D-2-(4-<2,4-Diazospiro[5,5]undec-2-ene-3-yl>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 4.27 g of 4-(2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid hydrochloride were reacted with 10 ml of thionyl chloride in a manner analogous to that described in Example 7(a). 4.08 g of 4-(2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid chloride-hydrochloride were obtained in the form of colourless crystals.

b. 4.1 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid and 4.0 g of 4-(2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid chloride-hydrochloride were reacted under the same conditions as described in Example 1(b). 5.6 g of the above-mentioned penicillin were obtained; decomposition point 212° to 214° C;  $R_f$ -value = 0.56.

## EXAMPLE 17

6-[D-2-(4-<9-Oxa-2,4-diazospiro[5,5]undec-2-ene-3-yl>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 3.25 g of 4-(9-oxa-2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid hydrochloride were reacted with 10 ml of thionyl chloride in a manner analogous to that described in Example 7(a). 3.4 g of 4-(9-oxa-2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid chloride-hydrochloride in the form of colourless crystals were obtained. Decomposition point 235° to 238° C.

b. 3.36 g of anhydrous 6-(D- $\alpha$ -aminophenylacetyl-amino)-penicillanic acid were reacted with 3.3 g of 4-(9-oxa-2,4-diazospiro[5,5]undec-2-ene-3-yl)-phenylacetic acid chloride-hydrochloride according to the method described in Example 1(b). 3.8 g of the above-specified penicillin were obtained.  $R_f$ -value = 0.36.

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## EXAMPLE 18

## 6-[D-2-(5-Amidino-2-thienylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

a. 7.36 g of 5-amidino-2-thienylacetic acid were stirred for 2 hours at room temperature with a mixture of 60 ml of anhydrous benzene and 15 ml of thionyl chloride. The 5-amidino-2-thienylacetic acid chloride-hydrochloride was filtered off with suction and washed with benzene and diethyl ether. After drying under reduced pressure, 9.0 g of the above-specified acid chloride-hydrochloride were obtained; decomposition point 158° to 160° C.

b. 12.35 g of anhydrous 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid and 8.36 g of 5-amidino-2-thienylacetic acid chloride-hydrochloride were reacted in a manner analogous to that described in Example 1(b). 10.7 g of the above-specified penicillin were obtained; decomposition point 199° to 200° C.  $R_f$ -value = 0.61.

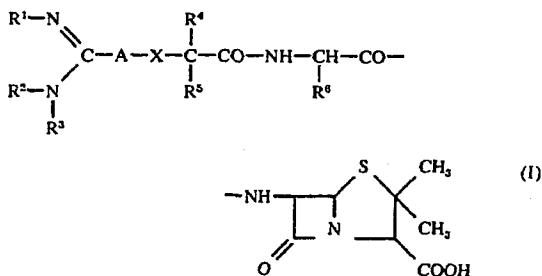
## EXAMPLE 19

## 6-[D-2-(4-amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid

2.2 g of trimethyl-chlorosilane were added dropwise, at 0° C, to a solution of 3.5 g of anhydrous 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid and 2.02 g of triethylamine in 60 ml of anhydrous methylene chloride. The reaction solution was stirred for 1 hour at 0° C and then combined at first with 2.02 g of triethylamine and subsequently with 2.5 g of 4-amidinophenoxyacetic acid chloride-hydrochloride. The reaction mixture was stirred for 1 hour at 0° C and for another hour at room temperature. After removal of the methylene chloride by distillation, the residue was triturated with 25 ml of water. The product that precipitated in the form of a powder was filtered off with suction, washed with water and dried under reduced pressure. In order to eliminate any triethylamine-hydrochloride and 6-(D- $\alpha$ -amino-phenylacetyl-amino)-penicillanic acid still present, the compound was treated in the same manner as described in Example 1(b) with a mixture of methylene chloride and triethylamine. 3.4 g of the above-specified penicillin were obtained. Decomposition point 198° to 200° C;  $R_f$ -value = 0.53.

We claim:

1. Acylaminopenicillanic acids of the general formula I



in which  $R^1$ ,  $R^2$  and  $R^3$  represent hydrogen or lower alkyl radicals which may be substituted by lower alkoxy and in which the radicals  $R^1$  and  $R^2$  or  $R^2$  and  $R^3$  may form together an alkylene radical of 2 to 4 carbon

14

atoms which may be interrupted by oxygen or nitrogen and which may be substituted by lower alkyl radicals which may be interrupted by oxygen and wherein these substituents of the alkylene ring may further be closed

to form a ring which may be interrupted by oxygen;  $R^4$  and  $R^5$  represent hydrogen or lower alkyl,  $R^6$  represents phenyl which may be substituted by hydroxyl, lower alkyl, lower alkoxy or halogen; dihydrophenyl; or thienyl, furyl or pyridyl which may be substituted by lower alkyl or lower alkoxy; A represents a benzene or thiophene ring which may be substituted by lower alkoxy, halogen or lower alkyl, and X represents oxygen or a single bond.

2. 6-[D,L-2-(4-Amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

3. 6-[D-2-(4-Amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

4. 6-[D-2-(4-Amidinophenoxyacetyl-amino)-2-(4-hydroxyphenyl)-acetyl-amino]-penicillanic acid.

5. 6-[D-2-(3-Amidinophenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

6. 6-[D-2-(4-Amidinophenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

7. 6-[D-2-(4-Amidino-2-methoxyphenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

8. 6-[D-2-(4-Amidinophenoxyisobutylamino)-2-phenylacetyl-amino]-penicillanic acid.

9. 6-[D-2-(4-<2-Imidazolyl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

10. 6-[D-2-(4-<1,4,5,6-Tetrahydropyrimide-2-yl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

11. 6-[D-2-(4-<5,5-Dimethyl-1,4,5,6-tetrahydropyrimide-2-yl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

12. 6-[D-2-(4-<9-Oxa-2,4-diazaspiro[5,5]undec-2-ene-3-yl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

13. 6-[D-2-(4-<1,4,5,6-Tetrahydropyrimide-2-yl>-phenylacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

14. 6-[D-2-(4-<N,N-Pentamethyleneamidino>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

15. 6-[D-2-(4-<3-Azabicyclo[3,3,1]nonane-3-ylcarbonimidoyl>-phenoxyacetyl-amino)-2-phenylacetyl-amino]-penicillanic acid.

16. A pharmaceutical composition active against bacterial infections and comprising a pharmaceutically acceptable carrier and an antibacterially effective amount of an acylaminopenicillanic acid of the general formula I

55 
$$\begin{array}{c}
 \text{R}^1\text{-N} \\
 \diagup \\
 \text{C}=\text{A-X-C} \begin{array}{l} \text{R}^4 \\ \text{R}^5 \end{array} \text{CO-NH-CH-CO-} \\
 \diagdown \\
 \text{R}^2\text{-N} \quad \text{R}^3
 \end{array}
 \begin{array}{c}
 \text{S} \\
 | \\
 \text{CH}_3 \\
 | \\
 \text{CH}_3 \\
 | \\
 \text{COOH}
 \end{array}
 \quad (I)$$

60 in which  $R^1$  to  $R^6$ , A and X have the meanings given above in claim 1.

\* \* \* \* \*

A-873

# EXHIBIT 24

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# **Guidance for Industry**

## **Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations**

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
March 2003  
BP**

**Revision 1**

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**A-874**

# **Guidance for Industry**

## **Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations**

*Additional copies are available from:  
Division of Drug Information, HFD-240  
Center for Drug Evaluation and Research  
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<http://www.fda.gov/cder/guidance/index.htm>*

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
March 2003  
BP**

**Revision 1**

**A-875**

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## **Guidance for Industry<sup>1</sup>**

### **BA and BE Studies for Orally Administered Drug Products — General Considerations**

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

#### **I. INTRODUCTION**

This guidance is intended to provide recommendations to sponsors and/or applicants planning to include bioavailability (BA) and bioequivalence (BE) information for orally administered drug products in investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and their supplements. This guidance contains advice on how to meet the BA and BE requirements set forth in part 320 (21 CFR part 320) as they apply to dosage forms intended for oral administration.<sup>2</sup> The guidance is also generally applicable to nonorally administered drug products where reliance on systemic exposure measures is suitable to document BA and BE (e.g., transdermal delivery systems and certain rectal and nasal drug products). We believe that the guidance will be useful for applicants planning to conduct BA and BE studies during the IND period for an NDA, BE studies intended for submission in an ANDA, and BE studies conducted in the postapproval period for certain changes in both NDAs and ANDAs.<sup>3</sup>

This guidance revises the October 2000 guidance. We have revised our recommendations regarding (1) study design and dissolution methods development, (2) comparisons of BA measures, (3) the definition of proportionality, and (4) waivers for bioequivalence studies. The guidance also makes other revisions for clarification. We believe that these revisions provide clear guidance to sponsors conducting BA and BE studies for orally administered drug products.

<sup>2</sup> These dosage forms include tablets, capsules, solutions, suspensions, conventional/immediate release, and modified (extended, delayed) release drug products.

<sup>3</sup> Other Agency guidances are available that consider specific scale-up and postapproval changes (SUPAC) for different types of drug products to help satisfy regulatory requirements in part 320 and § 314.70 (21 CFR 314.70).

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FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

## II. BACKGROUND

### A. General

Studies to measure BA and/or establish BE of a product are important elements in support of INDs, NDAs, ANDAs, and their supplements. As part of INDs and NDAs for orally administered drug products, BA studies focus on determining the process by which a drug is released from the oral dosage form and moves to the site of action. BA data provide an estimate of the fraction of the drug absorbed, as well as its subsequent distribution and elimination. BA can be generally documented by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time. The systemic exposure profile determined during clinical trials in the IND period can serve as a benchmark for subsequent BE studies.

Studies to establish BE between two products are important for certain changes before approval for a pioneer product in NDA and ANDA submissions and in the presence of certain postapproval changes in NDAs and ANDAs. In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product (RLD). For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product (see 21 CFR 320.1(e) and 320.23(b)).

Both BA and BE studies are required by regulations, depending on the type of application being submitted. Under § 314.94, BE information is required to ensure therapeutic equivalence between a pharmaceutically equivalent test drug product and a reference listed drug. Regulatory requirements for documentation of BA and BE are provided in part 320, which contains two subparts. Subpart A covers general provisions, while subpart B contains 18 sections delineating the following general BA/BE requirements:

- Requirements for submission of BA and BE data (§ 320.21)
- Criteria for waiver of an in vivo BA or BE study (§ 320.22)
- Basis for demonstrating in vivo BA or BE (§ 320.23)
- Types of evidence to establish BA or BE (§ 320.24)
- Guidelines for conduct of in vivo BA studies (§ 320.25)
- Guidelines on design of single-dose BA studies (§ 320.26)
- Guidelines on design of multiple-dose in vivo BA studies (§ 320.27)
- Correlations of BA with an acute pharmacological effect or clinical evidence (§ 320.28)

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- Analytical methods for an in vivo BA study (§ 320.29)
- Inquiries regarding BA and BE requirements and review of protocols by FDA (§ 320.30)
- Applicability of requirements regarding an IND application (§ 320.31)
- Procedures for establishing and amending a BE requirement (§ 320.32)
- Criteria and evidence to assess actual or potential BE problems (§ 320.33)
- Requirements for batch testing and certification by FDA (§ 320.34)
- Requirements for in vitro batch testing of each batch (§ 320.35)
- Requirements for maintenance of records of BE testing (§ 320.36)
- Retention of BA samples (§ 320.38)
- Retention of BE samples (§ 320.63)

**B. Bioavailability**

Bioavailability is defined in § 320.1 as:

the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

This definition focuses on the processes by which the active ingredients or moieties are released from an oral dosage form and move to the site of action.

From a pharmacokinetic perspective, BA data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the BA data for a solution, suspension, or intravenous dosage form (21 CFR 320.25(d)(2) and (3)). In addition, BA studies provide other useful pharmacokinetic information related to distribution, elimination, the effects of nutrients on absorption of the drug, dose proportionality, linearity in pharmacokinetics of the active moieties and, where appropriate, inactive moieties. BA data can also provide information indirectly about the properties of a drug substance before entry into the systemic circulation, such as permeability and the influence of presystemic enzymes and/or transporters (e.g., p-glycoprotein).

BA for orally administered drug products can be documented by developing a systemic exposure profile. A profile can be obtained by measuring the concentration of active ingredients and/or active moieties and, when appropriate, its active metabolites over time in samples collected from the systemic circulation. Systemic exposure patterns reflect both release of the drug substance from the drug product and a series of possible presystemic/systemic actions on the drug substance after its release from the drug product. We recommend that additional comparative studies be performed to understand the relative contribution of these processes to the systemic exposure pattern.

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One regulatory objective is to assess, through appropriately designed BA studies, the performance of the formulations used in the clinical trials that provide evidence of safety and efficacy (21 CFR 320.25(d)(1)). Before marketing a drug product, the performance of the clinical trial dosage form can be optimized, in the context of demonstrating safety and efficacy. The systemic exposure profiles of clinical trial material can be used as a benchmark for subsequent formulation changes and can be useful as a reference for future BE studies.

Although BA studies have many pharmacokinetic objectives beyond formulation performance as described above, we note that subsequent sections of this guidance focus on using relative BA (referred to as product quality BA) and, in particular, BE studies as a means to document product quality. In vivo performance, in terms of BA/BE, can be considered to be one aspect of product quality that provides a link to the performance of the drug product used in clinical trials and to the database containing evidence of safety and efficacy.

### **C. Bioequivalence**

Bioequivalence is defined in § 320.1 as:

the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

As noted in the statutory definitions, both BE and product quality BA focus on the release of a drug substance from a drug product and subsequent absorption into the systemic circulation. As a result, we recommend that similar approaches to measuring BA in an NDA generally be followed in demonstrating BE for an NDA or an ANDA. Establishing product quality BA is a benchmarking effort with comparisons to an oral solution, oral suspension, or an intravenous formulation. In contrast, demonstrating BE is usually a more formal comparative test that uses specified criteria for comparisons and predetermined BE limits for such criteria.

#### *1. IND/NDAs*

BE documentation can be useful during the IND or NDA period to establish links between (1) early and late clinical trial formulations; (2) formulations used in clinical trial and stability studies, if different; (3) clinical trial formulations and to-be-marketed drug product; and (4) other comparisons, as appropriate. In each comparison, the new formulation or new method of manufacture is the test product and the prior formulation or method of manufacture is the reference product. We recommend that the determination to redocument BE during the IND period be generally left to the judgment of the sponsor, who can wish to use the principles of relevant guidances (in this guidance, see sections II.C.3, Postapproval Changes, and III.D, in Vitro Studies) to determine when changes in components, composition, and/or method of manufacture suggest further in vitro and/or in vivo studies be performed.

A test product can fail to meet BE limits because the test product has higher or lower measures of rate and extent of absorption compared to the reference product or because

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the performance of the test or reference product is more variable. In some cases, nondocumentation of BE can arise because of inadequate numbers of subjects in the study relative to the magnitude of intrasubject variability, and not because of either high or low relative BA of the test product. Adequate design and execution of a BE study will facilitate understanding of the causes of nondocumentation of BE.

Where the test product generates plasma levels that are substantially above those of the reference product, the regulatory concern is not therapeutic failure, but the adequacy of the safety database from the test product. Where the test product has levels that are substantially below those of the reference product, the regulatory concern becomes therapeutic efficacy. When the variability of the test product rises, the regulatory concern relates to both safety and efficacy, because it may suggest that the test product does not perform as well as the reference product, and the test product may be too variable to be clinically useful.

Proper mapping of individual dose-response or concentration-response curves is useful in situations where the drug product has plasma levels that are either higher or lower than the reference product and are outside usual BE limits. In the absence of individual data, population dose-response or concentration-response data acquired over a range of doses, including doses above the recommended therapeutic doses, may be sufficient to demonstrate that the increase in plasma levels would not be accompanied by additional risk. Similarly, population dose- or concentration-response relationships observed over a lower range of doses, including doses below the recommended therapeutic doses, may be able to demonstrate that reduced levels of the test product compared to the reference product are associated with adequate efficacy. In either event, the burden is on the sponsor to demonstrate the adequacy of the clinical trial dose-response or concentration-response data to provide evidence of therapeutic equivalence. In the absence of this evidence, failure to document BE may suggest the product should be reformulated, the method of manufacture for the test product be changed, and/or the BE study be repeated.

## 2. *ANDAs*

BE studies are a critical component of ANDA submissions. The purpose of these studies is to demonstrate BE between a pharmaceutically equivalent generic drug product and the corresponding reference listed drug (21 CFR 314.94 (a)(7)). Together with the determination of pharmaceutical equivalence, establishing BE allows a regulatory conclusion of therapeutic equivalence.

## 3. *Postapproval Changes*

Information on the types of in vitro dissolution and in vivo BE studies that we recommend be conducted for immediate-release and modified-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes is provided in the FDA guidances for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence*

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*Documentation; and SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation.* In the presence of certain major changes in components, composition, and/or method of manufacture after approval, we recommend that in vivo BE be redemonstrated. For approved NDAs, we also recommend that the drug product after the change be compared to the drug product before the change. For approved ANDAs, we also recommend that the drug product after the change be compared to the reference listed drug. Under section 506A(c)(2)(B) of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 356a(c)(2)(B)), postapproval changes requiring completion of studies in accordance with part 320 must be submitted in a supplement and approved by FDA before distributing a drug product made with the change.

### III. METHODS TO DOCUMENT BA AND BE

As noted in § 320.24, several in vivo and in vitro methods can be used to measure product quality BA and to establish BE. In descending order of preference, these include pharmacokinetic, pharmacodynamic, clinical, and in vitro studies. These general approaches are discussed in the following sections of this guidance. Product quality BA and BE frequently rely on pharmacokinetic measures such as AUC and C<sub>max</sub> that are reflective of systemic exposure.

#### A. Pharmacokinetic Studies

##### 1. General Considerations

The statutory definitions of BA and BE, expressed in terms of rate and extent of absorption of the active ingredient or moiety to the site of action, emphasize the use of pharmacokinetic measures in an accessible biological matrix such as blood, plasma, and/or serum to indicate release of the drug substance from the drug product into the systemic circulation.<sup>4</sup> This approach rests on an understanding that measuring the active moiety or ingredient at the site of action is generally not possible and, furthermore, that some relationship exists between the efficacy/safety and concentration of active moiety and/or its important metabolite or metabolites in the systemic circulation. To measure product quality BA and establish BE, reliance on pharmacokinetic measurements may be viewed as a bioassay that assesses release of the drug substance from the drug product into the systemic circulation. A typical study is conducted as a crossover study. In this type of study, clearance, volume of distribution, and absorption, as determined by physiological variables (e.g. gastric emptying, motility, pH), are assumed to have less interoccasion variability compared to the variability arising from formulation performance. Therefore, differences between two products because of formulation factors can be determined.

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<sup>4</sup> If serial measurements of the drug or its metabolites in plasma, serum, or blood cannot be accomplished, measurement of urinary excretion can be used to document BE.

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#### 2. *Pilot Study*

If the sponsor chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full BE study. The study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information. For example, for conventional immediate-release products, careful timing of initial samples may avoid a subsequent finding in a full-scale study that the first sample collection occurs after the plasma concentration peak. For modified-release products, a pilot study can help determine the sampling schedule to assess lag time and dose dumping. A pilot study that documents BE can be appropriate, provided its design and execution are suitable and a sufficient number of subjects (e.g., 12) have completed the study.

#### 3. *Pivotal Bioequivalence Studies*

General recommendations for a standard BE study based on pharmacokinetic measurements are provided in Attachment A.

#### 4. *Study Designs*

Nonreplicate crossover study designs are recommended for BE studies of immediate-release and modified-release dosage forms. However, sponsors and/or applicants have the option of using replicate designs for BE studies for these drug products. Replicate study designs may offer several scientific advantages compared to nonreplicate designs. The advantages of replicate study designs may be that they (1) allow comparisons of within-subject variances for the test and reference products, (2) provide more information about the intrinsic factors underlying formulation performance, and (3) reduce the number of subjects participating in the BE study. The recommended method of analysis of nonreplicate or replicate studies to establish BE is average bioequivalence, as discussed in section IV. General recommendations for nonreplicate study designs are provided in Attachment A. Recommendations for replicate study designs can be found in the guidance for industry *Statistical Approaches to Establishing Bioequivalence*.

#### 5. *Study Population*

We recommend that, unless otherwise indicated by a specific guidance, subjects recruited for in vivo BE studies be 18 years of age or older and capable of giving informed consent. This guidance recommends that in vivo BE studies be conducted in individuals representative of the general population, taking into account age, sex, and race. We recommend that if the drug product is intended for use in both sexes, the sponsor attempt to include similar proportions of males and females in the study. If the drug product is to be used predominantly in the elderly, we also recommend that the sponsor attempt to include as many subjects of 60 years of age or older as possible. We recommend that the total number of subjects in the study provide adequate power for BE demonstration, but it is not expected that there will be sufficient power to draw conclusions for each subgroup.

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Statistical analysis of subgroups is not recommended. We recommend that restrictions on admission into the study generally be based solely on safety considerations. In some instances, it may be useful to admit patients into BE studies for whom a drug product is intended. In this situation, we recommend that sponsors and/or applicants attempt to enter patients whose disease process is stable for the duration of the BE study. In accordance with § 320.31, for some products that will be submitted in ANDAs, an IND may be required for BE studies to ensure patient safety.

6. *Single-Dose/Multiple-Dose Studies*

Instances where multiple-dose studies can be useful are defined under § 320.27(a)(3). However, this guidance generally recommends single-dose pharmacokinetic studies for both immediate- and modified-release drug products to demonstrate BE because they are *generally* more sensitive in assessing release of the drug substance from the drug product into the systemic circulation (see section V). We recommend that if a multiple-dose study design is important, appropriate dosage administration and sampling be carried out to document attainment of steady state.

7. *Bioanalytical Methodology*

We recommend sponsors ensure that bioanalytical methods for BA and BE studies are accurate, precise, selective, sensitive, and reproducible. A separate FDA guidance entitled *Bioanalytical Method Validation* is available to assist sponsors in validating bioanalytical methods.

8. *Pharmacokinetic Measures of Systemic Exposure*

Both direct (e.g., rate constant, rate profile) and indirect (e.g., C<sub>max</sub>, T<sub>max</sub>, mean absorption time, mean residence time, C<sub>max</sub> normalized to AUC) pharmacokinetic measures are limited in their ability to assess rate of absorption. This guidance, therefore, recommends a change in focus from these direct or indirect measures of absorption rate to measures of systemic exposure. C<sub>max</sub> and AUC can continue to be used as measures for product quality BA and BE, but more in terms of their capacity to assess exposure than their capacity to reflect rate and extent of absorption. We recommend that reliance on systemic exposure measures reflect comparable rate and extent of absorption, which in turn would achieve the underlying statutory and regulatory objective of ensuring comparable therapeutic effects. Exposure measures are defined relative to early, peak, and total portions of the plasma, serum, or blood concentration-time profile, as follows:

a. *Early Exposure*

For orally administered immediate-release drug products, BE can generally be demonstrated by measurements of peak and total exposure. An early exposure measure may be informative on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic/pharmacodynamic studies that call for better control of drug absorption into the systemic circulation (e.g., to ensure rapid onset of an

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analgesic effect or to avoid an excessive hypotensive action of an antihypertensive). In this setting, the guidance recommends use of partial AUC as an early exposure measure. We recommend that the partial area be truncated at the population median of  $T_{max}$  values for the reference formulation. We also recommend that at least two quantifiable samples be collected before the expected peak time to allow adequate estimation of the partial area.

b. Peak Exposure

We recommend that peak exposure be assessed by measuring the peak drug concentration ( $C_{max}$ ) obtained directly from the data without interpolation.

c. Total Exposure

For single-dose studies, we recommend that the measurement of total exposure be:

- Area under the plasma/serum/blood concentration-time curve from time zero to time  $t$  ( $AUC_{0-t}$ ), where  $t$  is the last time point with measurable concentration for individual formulation.
- Area under the plasma/serum/blood concentration-time curve from time zero to time infinity ( $AUC_{0-\infty}$ ), where  $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$ ,  $C_t$  is the last measurable drug concentration and  $\lambda_z$  is the terminal or elimination rate constant calculated according to an appropriate method. We recommend that the terminal half-life ( $t_{1/2}$ ) of the drug also be reported.

For steady-state studies, we recommend that the measurement of total exposure be the area under the plasma, serum, or blood concentration-time curve from time zero to time  $\tau$  over a dosing interval at steady state ( $AUC_{0-\tau}$ ), where  $\tau$  is the length of the dosing interval.

## B. Pharmacodynamic Studies

Pharmacodynamic studies are not recommended for orally administered drug products when the drug is absorbed into the systemic circulation and a pharmacokinetic approach can be used to assess systemic exposure and establish BE. However, in those instances where a pharmacokinetic approach is not possible, suitably validated pharmacodynamic methods can be used to demonstrate BE.

## C. Comparative Clinical Studies

Where there are no other means, well-controlled clinical trials in humans can be useful to provide supportive evidence of BA or BE. However, we recommend that the use of comparative clinical trials as an approach to demonstrate BE generally be considered insensitive and be avoided where possible (21 CFR 320.24). The use of BE studies with clinical trial endpoints can

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be appropriate to demonstrate BE for orally administered drug products when measurement of the active ingredients or active moieties in an accessible biological fluid (pharmacokinetic approach) or pharmacodynamic approach is infeasible.

#### **D. In Vitro Studies**

Under certain circumstances, product quality BA and BE can be documented using in vitro approaches (21 CFR 320.24(b)(5) and 21 CFR 320.22(d)(3)). For highly soluble, highly permeable, rapidly dissolving, and orally administered drug products, documentation of BE using an in vitro approach (dissolution studies) is appropriate based on the biopharmaceutics classification system.<sup>5</sup> This approach may also be suitable under some circumstances in assessing BE during the IND period, for NDA and ANDA submissions, and in the presence of certain postapproval changes to approved NDAs and ANDAs. In addition, in vitro approaches to documenting BE for *nonbioproblem* drugs approved before 1962 remain appropriate (21 CFR 320.33).

Dissolution testing is also used to assess batch-to-batch quality, where the dissolution tests, with defined procedures and acceptance criteria, are used to allow batch release. We recommend that dissolution testing is also used to (1) provide process control and quality assurance, and (2) assess whether further BE studies relative to minor postapproval changes be conducted, where dissolution can function as a signal of bioinequivalence. In vitro dissolution characterization is encouraged for all product formulations investigated (including prototype formulations), particularly if in vivo absorption characteristics are being defined for the different product formulations. Such efforts may enable the establishment of an in vitro-in vivo correlation. When an in vitro-in vivo correlation or association is available (21 CFR 320.24(b)(1)(ii)), the in vitro test can serve not only as a quality control specification for the manufacturing process, but also as an indicator of how the product will perform in vivo. The following guidances provide recommendations on the development of dissolution methodology, setting specifications, and the regulatory applications of dissolution testing: (1) *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*; and (2) *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations*.

We recommend that the following information generally be included in the dissolution method development report for solid oral dosage forms:

For an NDA:

- The pH solubility profile of the drug substance
- Dissolution profiles generated at different agitation speeds (e.g., 100 to 150 revolutions per minute (rpm) for U.S. Pharmacopeia (USP) Apparatus I (basket), or 50 to 100 rpm for USP Apparatus II (paddle))

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<sup>5</sup> See the FDA guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*. This document provides complementary information on the Biopharmaceutics Classification System (BCS).

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- Dissolution profiles generated on all strengths in at least three dissolution media (e.g., pH 1.2, 4.5, and 6.8 buffer). Water can be used as an additional medium. If the drug being considered is poorly soluble, appropriate concentrations of surfactants are recommended.

It is recommended that the sponsor select the agitation speed and medium that provide adequate discriminating ability, taking into account all the available in vitro and in vivo data.

For ANDAs:

- For immediate-release drug products, we recommend that the appropriate USP method be submitted. If there is no USP method available, we recommend that the FDA method for the reference listed drug be used. If the USP and/or FDA methods are not available, we recommend that the dissolution method development report described above be submitted.
- For modified-release products, dissolution profiles using the appropriate USP method (if available) can be submitted. If there is no USP method available, we recommend that the FDA method for the reference listed drug be used. In addition, we recommend that profiles using at least three other dissolution media (e.g., pH 1.2, 4.5, and 6.8 buffer) and water be submitted.

This guidance recommends that dissolution data from three batches for both NDAs and ANDAs be used to set dissolution specifications for modified-release dosage forms, including extended-release dosage forms.

## **IV. COMPARISON OF BA MEASURES IN BE STUDIES**

An equivalence approach has been and continues to be recommended for BE comparisons. The recommended approach relies on (1) a criterion to allow the comparison, (2) a confidence interval for the criterion, and (3) a BE limit. Log-transformation of exposure measures before statistical analysis is recommended. BE studies are performed as single-dose, crossover studies. To compare measures in these studies, data have been analyzed using an average BE criterion. This guidance recommends continued use of an average BE criterion to compare BA measures for replicate and nonreplicate BE studies of both immediate- and modified-release products.

## **V. DOCUMENTATION OF BA AND BE**

An in vivo study is generally recommended for all solid oral dosage forms approved after 1962 and for *bioproblem* drug products approved before 1962. Waiver of in vivo studies for different strengths of a drug product can be granted under § 320.22(d)(2) when (1) the drug product is in the same dosage form, but in a different strength; (2) this different strength is *proportionally similar* in its active and inactive ingredients to the strength of the product for which the same manufacturer has conducted an appropriate in vivo study; and (3) the new strength meets an

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appropriate in vitro dissolution test. This guidance defines *proportionally similar* in the following ways:

- All active and inactive ingredients are in exactly the same proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients, exactly half that of a tablet of 100-mg strength, and twice that of a tablet of 25-mg strength).
- Active and inactive ingredients are not in exactly the same proportion between different strengths as stated above, but the ratios of inactive ingredients to total weight of the dosage form are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II.
- For high potency drug substances, where the amount of the active drug substance in the dosage form is relatively low, the total weight of the dosage form remains nearly the same for all strengths (within  $\pm 10\%$  of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients. The changes in the inactive ingredients are within the limits defined by the SUPAC-IR and SUPAC-MR guidances up to and including Level II.

Exceptions to the above definitions may be possible, if adequate justification is provided.

**A. Solutions**

For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, in vivo BA and/or BE can be waived (21 CFR 320.22(b)(3)(i)). Generally, in vivo BE studies are waived for solutions on the assumption that release of the drug substance from the drug product is self-evident and that the solutions do not contain any excipient that significantly affects drug absorption (21 CFR 320.22(b)(3)(iii)). However, there are certain excipients, such as sorbitol or mannitol, that can reduce the bioavailability of drugs with low intestinal permeability in amounts sometimes used in oral liquid dosage forms.

**B. Suspensions**

We recommend that BA and BE for a suspension generally be established for immediate-release solid oral dosage forms, and both in vivo and in vitro studies are recommended.

**C. Immediate-Release Products: Capsules and Tablets****1. General Recommendations**

For product quality BA and BE studies, we recommend that where the focus is on release of the drug substance from the drug product into the systemic circulation, a single-dose, fasting study be performed. We also recommend that in vivo BE studies be accompanied

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by in vitro dissolution profiles on all strengths of each product. For ANDAs, we also recommend that the BE study be conducted between the test product and reference listed drug using the strength(s) specified in *Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book)*.

2. *Waivers of In Vivo BE Studies (Biowaivers)*

a. INDs, NDAs, and ANDAs: Preapproval

When the drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to the strength on which BA or BE testing has been conducted, an in vivo BE demonstration of one or more lower strengths can be waived based on dissolution tests and an in vivo study on the highest strength.<sup>8</sup>

For an NDA, biowaivers of a higher strength will be determined to be appropriate based on (1) clinical safety and/or efficacy studies including data on the dose and the desirability of the higher strength, (2) linear elimination kinetics over the therapeutic dose range, (3) the higher strength being proportionally similar to the lower strength, and (4) the same dissolution procedures being used for both strengths and similar dissolution results obtained. We recommend that a dissolution profile be generated for all strengths.

If an appropriate dissolution method has been established (see section III.D.), and the dissolution results indicate that the dissolution characteristics of the product are not dependent on the product strength, then dissolution profiles in one medium are usually sufficient to support waivers of in vivo testing. Otherwise, dissolution data in three media (pH 1.2, 4.5, and 6.8) are recommended. We recommend that the  $f_2$  test be used to compare profiles from the different strengths of the product. An  $f_2$  value  $\geq 50$  indicates a sufficiently similar dissolution profile such that further in vivo studies are not needed. For an  $f_2$  value  $< 50$ , further discussions with CDER review staff may help to determine whether an in vivo study is appropriate (21 CFR 320.22(d)(2)(ii)). The  $f_2$  approach is not suitable for rapidly dissolving drug products (e.g.,  $\geq 85\%$  dissolved in 15 minutes or less).

For an ANDA, conducting an in vivo study on a strength that is not the highest may be appropriate for reasons of safety, subject to approval by the Division of Bioequivalence, Office of Generic Drugs, and provided that the following conditions are met:

- Linear elimination kinetics has been shown over the therapeutic dose range.

<sup>8</sup> This recommendation modifies a prior policy of allowing biowaivers for only three lower strengths on ANDAs.

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- The higher strengths of the test and reference products are proportionally similar to their corresponding lower strength.
- Comparative dissolution testing on the higher strength of the test and reference products is submitted and found to be appropriate.

b. NDAs and ANDAs: Postapproval

Information on the types of in vitro dissolution and in vivo BE studies for immediate-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes are provided in an FDA guidance for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. For postapproval changes, we recommend that the in vitro comparison be made between the prechange and postchange products. In instances where dissolution profile comparisons are suggested, we also recommend an  $f_2$  test be used. An  $f_2$  value of  $\geq 50$  suggests a sufficiently similar dissolution profile and no further in vivo studies are needed. When in vivo BE studies are called for, we recommend that the comparison be made for NDAs between the prechange and postchange products, and for ANDAs between the postchange and reference listed drug products.

**D. Modified-Release Products**

Modified-release products include delayed-release products and extended- (controlled) release products.

As defined in the USP, delayed-release drug products are dosage forms that release the drugs at a time later than immediately after administration (i.e., these drug products exhibit a lag time in quantifiable plasma concentrations). Typically, coatings (e.g., enteric coatings) are intended to delay the release of medication until the dosage form has passed through the acidic medium of the stomach. In vivo tests for delayed-release drug products are similar to those for extended-release drug products. We recommend that in vitro dissolution tests for these products document that they are stable under acidic conditions and that they release the drug only in a neutral medium (e.g., pH 6.8).

Extended-release drug products are dosage forms that allow a reduction in dosing frequency as compared to when the drug is present in an immediate-release dosage form. These drug products can be developed to reduce fluctuations in plasma concentrations. Extended-release products can be capsules, tablets, granules, pellets, and suspensions. If any part of a drug product includes an extended-release component, the following recommendations apply.

1. NDAs: BA and BE Studies

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An NDA can be submitted for a previously unapproved new molecular entity, new salt, new ester, prodrug, or other noncovalent derivative of a previously approved new molecular entity formulated as a modified-release drug product. We recommend that the first modified-release drug product for a previously approved immediate-release drug product be submitted as an NDA. We also recommend that subsequent modified-release products that are pharmaceutically equivalent and bioequivalent to the listed drug product be submitted as ANDAs. BA requirements for the NDA of an extended-release product are listed in § 320.25(f). The purpose of an in vivo BA study for which a controlled-release claim is made is to determine if all of the following conditions are met:

- The drug product meets the controlled-release claims made for it.
- The BA profile established for the drug product rules out the occurrence of any dose dumping.
- The drug product's steady-state performance is equivalent to a currently marketed noncontrolled release or controlled-release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full NDA.
- The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.

As noted in § 320.25(f)(2), "the reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the controlled release claims made for the drug product," such as:

- A solution or suspension of the active drug ingredient or therapeutic moiety
- A currently marketed noncontrolled-release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling
- A currently marketed controlled-release drug product subject to an approved full NDA containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling

This guidance recommends that the following BA studies be conducted for an extended-release drug product submitted as an NDA:

- A single-dose, fasting study on all strengths of tablets and capsules and highest strength of beaded capsules
- A single-dose, food-effect study on the highest strength

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- A steady-state study on the highest strength

BE studies are recommended when substantial changes in the components or composition and/or method of manufacture for an extended-release drug product occur between the to-be-marketed NDA dosage form and the clinical trial material.

## 2. *ANDAs: BE Studies*

For modified-release products submitted as ANDAs, the following studies are recommended: (1) a single-dose, nonreplicate, fasting study comparing the highest strength of the test and reference listed drug product and (2) a food-effect, nonreplicate study comparing the highest strength of the test and reference product (see section VI.A). Because single-dose studies are considered more sensitive in addressing the primary question of BE (i.e., release of the drug substance from the drug product into the systemic circulation), multiple-dose studies are generally not recommended, even in instances where nonlinear kinetics are present.

## 3. *Waivers of In Vivo BE Studies (Biowaivers): NDAs and ANDAs*

### a. Beaded Capsules — Lower Strength

We recommend that for modified-release beaded capsules where the strength differs only in the number of beads containing the active moiety, a single-dose, fasting BE study be carried out only on the highest strength, with waiver of in vivo studies for lower strengths based on dissolution profiles. A dissolution profile can be generated for each strength using the recommended dissolution method. The  $f_2$  test can be used to compare profiles from the different strengths of the product. An  $f_2$  value of  $\geq 50$  can be used to confirm that further in vivo studies are not needed.

### b. Tablets — Lower Strength

For modified-release tablets, when the drug product is in the same dosage form but in a different strength, when it is proportionally similar in its active and inactive ingredients, and when it has the same drug release mechanism, an in vivo BE determination of one or more lower strengths can be waived based on dissolution profile comparisons, with an in vivo study only on the highest strength. We recommend that the drug products exhibit similar dissolution profiles between the highest strength and the lower strengths based on the  $f_2$  test in at least three dissolution media (e.g., pH 1.2, 4.5 and 6.8). We recommend that the dissolution profile be generated on the test and reference products of all strengths.

## 4. *Postapproval Changes*

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Information on the types of in vitro dissolution and in vivo BE studies for extended-release drug products approved as either NDAs or ANDAs in the presence of specified postapproval changes are provided in an FDA guidance for industry entitled *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. We recommend that for postapproval changes, the in vitro comparison be made between the prechange and postchange products. In instances where dissolution profile comparisons are recommended, an  $f_2$  test can be used. An  $f_2$  value of  $\geq 50$  suggests a similar dissolution profile. A failure to demonstrate similar dissolution profiles may indicate an in vivo BE study be performed. When in vivo BE studies are conducted, we recommend that the comparison be made for NDAs between the prechange and postchange products, and for ANDAs between the postchange product and reference listed drug.

#### **E. Miscellaneous Dosage Forms**

We recommend that rapidly dissolving drug products, such as buccal and sublingual dosage forms (and chewable tablets), be tested for in vitro dissolution and in vivo BA and/or BE. We recommend that chewable tablets (as a whole) be subject to in vitro dissolution testing because they might be swallowed by a patient without proper chewing. In general, we recommend that in vitro dissolution test conditions for chewable tablets be the same as for nonchewable tablets of the same active ingredient or moiety. Infrequently, different test conditions or acceptance criteria can be indicated for chewable and nonchewable tablets, but we recommend these differences, if they exist, be resolved with the appropriate review division.

## **VI. SPECIAL TOPICS**

### **A. Food-Effect Studies**

Co-administration of food with oral drug products may influence drug BA and/or BE. Food-effect BA studies focus on the effects of food on the release of the drug substance from the drug product as well as the absorption of the drug substance. BE studies with food focus on demonstrating comparable BA between test and reference products when coadministered with meals. Usually, a single-dose, two-period, two-treatment, two-sequence crossover study is recommended for both food-effect BA and BE studies.

### **B. Moieties to Be Measured**

#### *1. Parent Drug Versus Metabolites*

The moieties to be measured in biological fluids collected in BA and BE studies are either the active drug ingredient or its active moiety in the administered dosage form

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(parent drug) and, when appropriate, its active metabolites (21 CFR 320.24(b)(1)(i)).<sup>9</sup> This guidance recommends the following approaches for BA and BE studies.

For BA studies (see section II.B), we recommend that determination of moieties to be measured in biological fluids take into account both concentration and activity.

*Concentration* refers to the relative quantity of the parent drug or one or more metabolites in a given volume of an accessible biological fluid such as blood or plasma. *Activity* refers to the relative contribution of the parent drug and its metabolite(s) in the biological fluids to the clinical safety and/or efficacy of the drug. For BA studies, we also recommend that both the parent drug and its major active metabolites be measured, if analytically feasible.

For BE studies, measurement of only the parent drug released from the dosage form, rather than the metabolite, is generally recommended. The rationale for this recommendation is that concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are exceptions to this general approach.

- Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time. We recommend that the metabolite data obtained from these studies be subject to a confidence interval approach for BE demonstration. If there is a clinical concern related to efficacy or safety for the parent drug, we also recommend that sponsors and/or applicants contact the appropriate review division to determine whether the parent drug should be measured and analyzed statistically.
- A metabolite may be formed as a result of gut wall or other presystemic metabolism. If the metabolite contributes meaningfully to safety and/or efficacy, we also recommend that the metabolite and the parent drug be measured. When the relative activity of the metabolite is low and does not contribute meaningfully to safety and/or efficacy, it does not have to be measured. We recommend that the parent drug measured in these BE studies be analyzed using a confidence interval approach. The metabolite data can be used to provide supportive evidence of comparable therapeutic outcome.

## 2. *Enantiomers Versus Racemates*

For BA studies, measurement of individual enantiomers may be important. For BE studies, this guidance recommends measurement of the racemate using an achiral assay. Measurement of individual enantiomers in BE studies is recommended only when all of

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<sup>9</sup> A dosage form contains active and, usually, inactive ingredients. The active ingredient may be a prodrug that becomes active with further in vivo transformation. An active moiety is the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt, or other noncovalent derivative of the molecule, responsible for the physiological or pharmacological action of the drug substance.

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the following conditions are met: (1) the enantiomers exhibit different pharmacodynamic characteristics, (2) the enantiomers exhibit different pharmacokinetic characteristics, (3) primary efficacy and safety activity resides with the minor enantiomer, and (4) nonlinear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such cases, we recommend that BE factors be applied to the enantiomers separately.

#### *3. Drug Products With Complex Mixtures as the Active Ingredients*

Certain drug products may contain complex drug substances (i.e., active moieties or active ingredients that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex drug substances cannot be characterized with regard to chemical structure and/or biological activity. Quantification of all active or potentially active components in pharmacokinetic studies to document BA and BE is neither encouraged nor desirable. Rather, we recommend that BA and BE studies be based on a small number of markers of rate and extent of absorption. Although a case-by-case determination, criteria for marker selection include amount of the moiety in the dosage form, plasma or blood levels of the moiety, and biological activity of the moiety relative to other moieties in the complex mixture. Where pharmacokinetic approaches are infeasible to assess rate and extent of absorption of a drug substance from a drug product, in vitro approaches may be preferred. Pharmacodynamic or clinical approaches may be called for if no quantifiable moieties are available for in vivo pharmacokinetic or in vitro studies.

#### **C. Long Half-Life Drugs**

In a BA or pharmacokinetic study involving an oral product with a long half-life drug, adequate characterization of the half-life calls for blood sampling over a long period of time. For a BE determination of an oral product with a long half-life drug, a nonreplicate, single-dose, crossover study can be conducted, provided an adequate washout period is used. If the crossover study is problematic, a BE study with a parallel design can be used. For either a crossover or parallel study, we recommend that sample collection time be adequate to ensure completion of gastrointestinal transit (approximately 2 to 3 days) of the drug product and absorption of the drug substance.  $C_{max}$  and a suitably truncated AUC can be used to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours ( $AUC_{0-72 \text{ hr}}$ ) can be used in place of  $AUC_{0-t}$  or  $AUC_{0-\infty}$ . For drugs demonstrating high intrasubject variability in distribution and clearance, AUC truncation warrants caution. In such cases, we also recommend that sponsors and/or applicants consult the appropriate review staff.

#### **D. First Point $C_{max}$**

The first point of a concentration-time curve in a BE study based on blood and/or plasma measurements is sometimes the highest point, which raises a question about the measurement of true  $C_{max}$  because of insufficient early sampling times. A carefully conducted pilot study may avoid this problem. Collection of an early time point between 5 and 15 minutes after dosing

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followed by additional sample collections (e.g., two to five) in the first hour after dosing may be sufficient to assess early peak concentrations. If this sampling approach is followed, we recommend that data sets be considered adequate, even when the highest observed concentration occurs at the first time point.

**E. Orally Administered Drugs Intended for Local Action**

Documentation of product quality BA for NDAs where the drug substance produces its effects by local action in the gastrointestinal tract can be achieved using clinical efficacy and safety studies and/or suitably designed and validated in vitro studies. Similarly, documentation of BE for ANDAs, and for both NDAs and ANDAs in the presence of certain postapproval changes, can be achieved using BE studies with clinical efficacy and safety endpoints and/or suitably designed and validated in vitro studies, if the latter studies are either reflective of important clinical effects or are more sensitive to changes in product performance compared to a clinical study. To ensure comparable safety, additional studies with and without food may help to understand the degree of systemic exposure that occurs following administration of a drug product intended for local action in the gastrointestinal tract.

**F. Narrow Therapeutic Range Drugs**

This guidance defines *narrow therapeutic range*<sup>10</sup> drug products as containing certain drug substances subject to therapeutic drug concentration or pharmacodynamic monitoring, and/or where product labeling indicates a narrow therapeutic range designation. Examples include digoxin, lithium, phenytoin, theophylline, and warfarin. Because not all drugs subject to therapeutic drug concentration or pharmacodynamic monitoring are narrow therapeutic range drugs, sponsors and/or applicants can contact the appropriate review division at CDER to determine whether a drug can or cannot be considered to have a narrow therapeutic range.

This guidance recommends that sponsors consider additional testing and/or controls to ensure the quality of drug products containing narrow therapeutic range drugs. The approach is designed to provide increased assurance of interchangeability for drug products containing specified narrow therapeutic range drugs. It is not designed to influence the practice of medicine or pharmacy.

Unless otherwise indicated by a specific guidance, this guidance recommends that the traditional BE limit of 80 to 125 percent for non-narrow therapeutic range drugs remain unchanged for the bioavailability measures (AUC and Cmax) of narrow therapeutic range drugs.

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<sup>10</sup> This guidance uses the term *narrow therapeutic range* instead of *narrow therapeutic index* drug, although the latter is more commonly used.

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**ATTACHMENT: GENERAL PHARMACOKINETIC STUDY DESIGN  
AND DATA HANDLING**

For both replicate and nonreplicate, in vivo pharmacokinetic BE studies, the following general approaches are recommended, recognizing that the elements can be adjusted for certain drug substances and drug products.

Study conduct:

- The test or reference products can be administered with about 8 ounces (240 milliliters) of water to an appropriate number of subjects under fasting conditions, unless the study is a food-effect BA and BE study.
- Generally, the highest marketed strength can be administered as a single unit. If warranted for analytical reasons, multiple units of the highest strength can be administered, providing the total single-dose remains within the labeled dose range.
- An adequate washout period (e.g., more than 5 half lives of the moieties to be measured) would separate each treatment.
- The lot numbers of both test and reference listed products and the expiration date for the reference product would be stated. The drug content of the test product cannot differ from that of the reference listed product by more than 5 percent. The sponsor can include a statement of the composition of the test product and, if possible, a side-by-side comparison of the compositions of test and reference listed products. In accordance with § 320.38, samples of the test and reference listed product must be retained for 5 years.
- Before and during each study phase, we recommend that subjects (1) be allowed water as desired except for 1 hour before and after drug administration, (2) be provided standard meals no less than 4 hours after drug administration, and (3) abstain from alcohol for 24 hours before each study period and until after the last sample from each period is collected.

Sample collection and sampling times:

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- We recommend that under normal circumstances, blood, rather than urine or tissue, be used. In most cases, drug, or metabolites are measured in serum or plasma. However, in certain cases, whole blood may be more appropriate for analysis. We recommend that blood samples be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, we recommend that 12 to 18 samples, including a predose sample, be collected per subject per dose. This sampling can continue for at least three or more terminal half lives of the drug. The exact timing for sample collection depends on the nature of the drug and the input from the administered dosage form. The sample collection can be spaced in such a way that the maximum concentration of the drug in the blood ( $C_{max}$ ) and terminal elimination rate constant ( $\lambda_z$ ) can be estimated accurately. At least three to four samples can be obtained during the terminal log-linear phase to obtain an accurate estimate of  $\lambda_z$  from linear regression. We recommend that the actual clock time when samples are drawn as well as the elapsed time related to drug administration be recorded.

Subjects with predose plasma concentrations:

- If the predose concentration is  $\leq 5$  percent of  $C_{max}$  value in that subject, the subject's data without any adjustments can be included in all pharmacokinetic measurements and calculations. We recommend that if the predose value is  $>$  than 5 percent of  $C_{max}$ , the subject be dropped from all BE study evaluations.

Data deletion due to vomiting:

- We recommend that data from subjects who experience emesis during the course of a BE study for immediate-release products be deleted from statistical analysis if vomiting occurs at or before 2 times median  $T_{max}$ . In the case of modified-release products, the data from subjects who experience emesis any time during the labeled dosing interval can be deleted.

The following pharmacokinetic information is recommended for submission:

- Plasma concentrations and time points
- Subject, period, sequence, treatment
- $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$ ,  $T_{max}$ ,  $\lambda_z$ , and  $t_{1/2}$
- Intersubject, intrasubject, and/or total variability, if available
- $C_{min}$  (concentration at the end of a dosing interval),  $C_{av}$  (average concentration during a dosing interval), degree of fluctuation  $[(C_{max}-C_{min})/C_{av}]$ , and swing  $[(C_{max}-C_{min})/C_{min}]$  if steady-state studies are employed
- Partial AUC, requested only as discussed in section III. A.9.a.

In addition, we recommend that the following statistical information be provided for  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , and  $C_{max}$ :

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- Geometric mean
- Arithmetic mean
- Ratio of means
- Confidence intervals

We also recommend that logarithmic transformation be provided for measures used for BE demonstration.

Rounding off of confidence interval values:

- We recommend that confidence interval (CI) values not be rounded off; therefore, to pass a CI limit of 80 to 125, the value would be at least 80.00 and not more than 125.00.